

Science Together



AZURA® Preparative HPLC

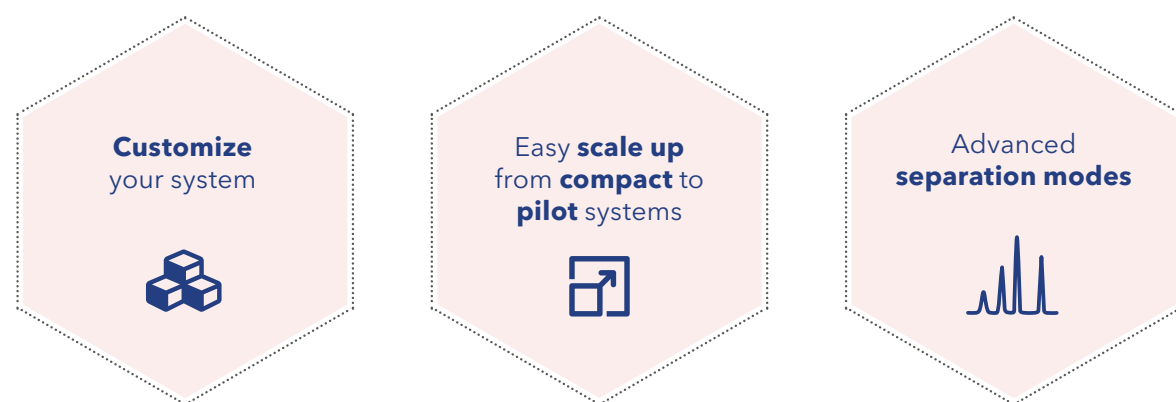
Flexible purification solutions



AZURA® Preparative HPLC

Customized purification

AZURA® preparative systems are the perfect solution for frequently changing separation tasks - from milligram to kilogram scale. Design your AZURA preparative system to your needs and combine flexibility and reliability.



AZURA® Prep systems are tailor-made for you. Configure your system from injection to detection and choose between different materials, flow rates, valves and detectors.

Due to the flexible design of our devices, you can easily change parts like pump heads or flow cells and integrate all components of the compact into the pilot-scale system.

AZURA® Prep systems can be used for special separation modes like peak recycling and stacked injections. We help you to configure your system and choose the best software for you.

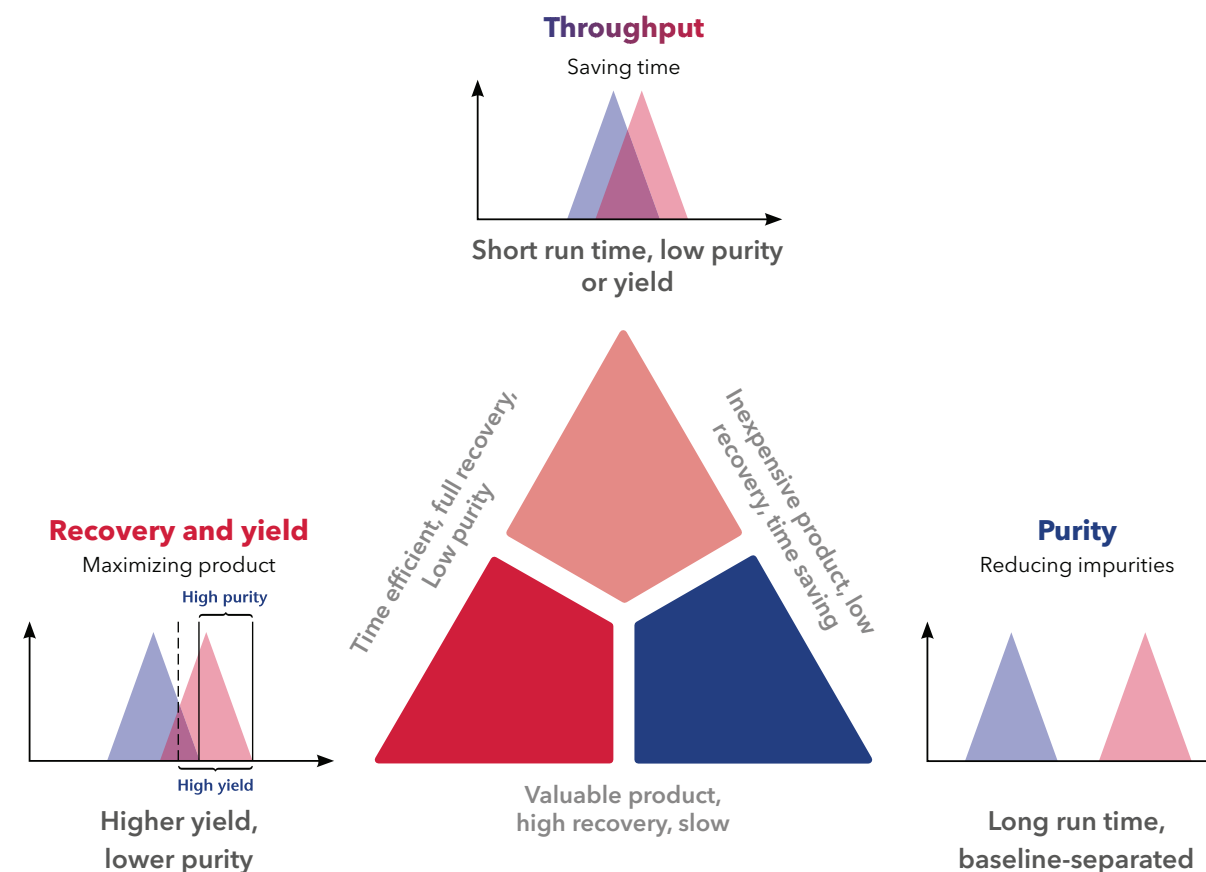
Preparative chromatography

The general objective of preparative chromatography is to isolate, purify and collect your target compounds. Preparative applications are often initially performed on an analytical level and need to be upscaled. Depending on the desired scale, the requirements for a preparative system differ in eluent supply, sample injection, column, and detection. We customize our systems to meet your chromatography scale-up and purification challenges. Benefit from our experience in preparative chromatography.

For more information: www.knauer.net/prep

Purification strategy: Prioritize purity, throughput or yield?

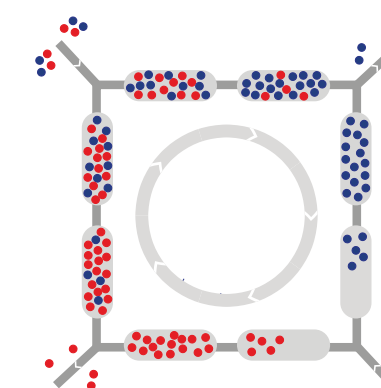
The dependencies between throughput, purity and yield always have to be considered in HPLC purifications. Whatever priority you decide for, with the AZURA preparative systems you can successfully adapt.



High purity and high yield with continuous chromatography

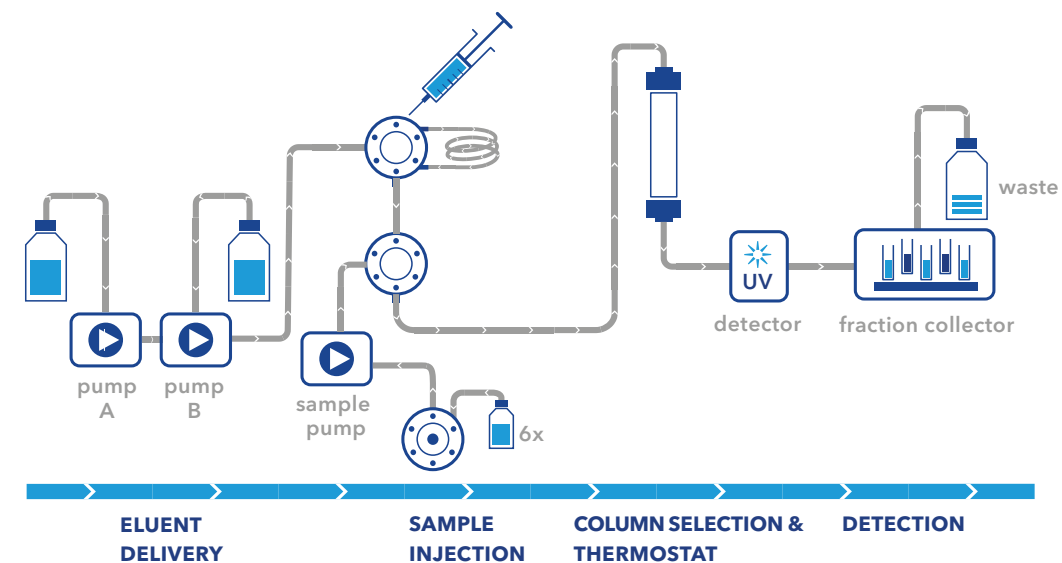
The AZURA SMB systems are the solution for your continuous purification task. Get higher productivity and purity than with comparable batch systems. Save up to 90 % of the solvent and reduce the solid phase costs up to 80 %.

For more information see page 32.



Scheme of SMB principle

Flexibility and performance



Assistant ○ Customizable combination of valves, detectors and pumps in one housing e.g. for column switching (see page 10)

Multi Column Base ○ Securely position up to three preparative columns, customize and organize your system with a wide range of accessories (see page 24)

HPLC column ○ Preparative separation columns (see page 15)

Fiber optics flow cell ○ Measure close to the column to minimize peak broadening with fiber optics (see page 17)

○ **Sample selection and injection**
Manual or automated injection: use a valve or a valve with feed pump (see page 11 + 12)

○ **Mobile Control**
Optional touch display and control tool for AZURA systems (see page 27)

○ **Detection**
Various detector types (UV/VIS, DAD, RI, FL, MS) and a selection of flow cells for a wide range of flow rates (see page 16)

○ **Fraction collection**
Fractionation valve or fraction collector for various flow rates (up to 1000 ml/min) (see page 20)

○ **Eluent delivery**
A choice of pumps with pump heads available in different materials allows maximum flow rates from 50 - 1000 ml/min. Isocratic, low and high pressure gradient forming are possible (see page 8)

Most flexible system solutions on the market

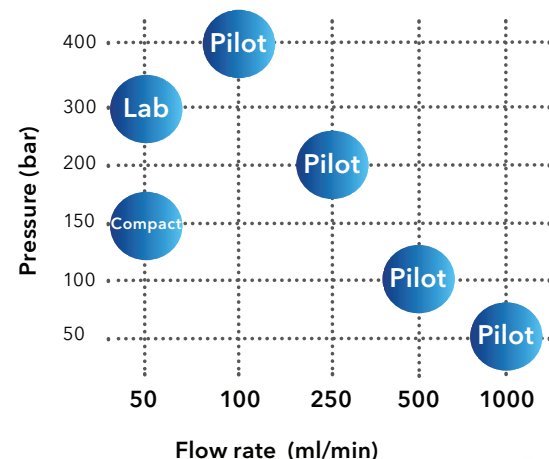
High or low pressure gradient 50 - 1000 ml/min

User friendly and powerful software

AZURA® Preparative HPLC

Upscaling from compact to pilot

The modular AZURA Preparative HPLC platform offers you the opportunity to build a purification system best suited to your needs.



AZURA® Compact Prep LC
Flow rate max. 50 ml/min*
Isocratic



AZURA® Lab Prep LC
Flow rate max. 50 ml/min*
Isocratic/HPG



AZURA® Pilot Prep LC
Flow rate max. 1000 ml/min*
Isocratic/LPG/HPG

AZURA system	Available pump heads Max. flow rate in ml/min*					Gradient options	
	50	100	250	500	1000	LPG low pressure	HPG high pressure
AZURA Compact Prep LC	•						
AZURA Lab Prep LC	•						•
AZURA Pilot Prep LC		•	•	•	•	•	•

* Information on best working conditions on pages 8-9.

Scale-up from compact to pilot

The AZURA Pilot Prep LC is the ideal solution for your upscaling tasks. The 100 ml pump head allows you to run your system under analytical conditions before adapting your method to preparative scale.

For more information:
www.knauer.net/prep

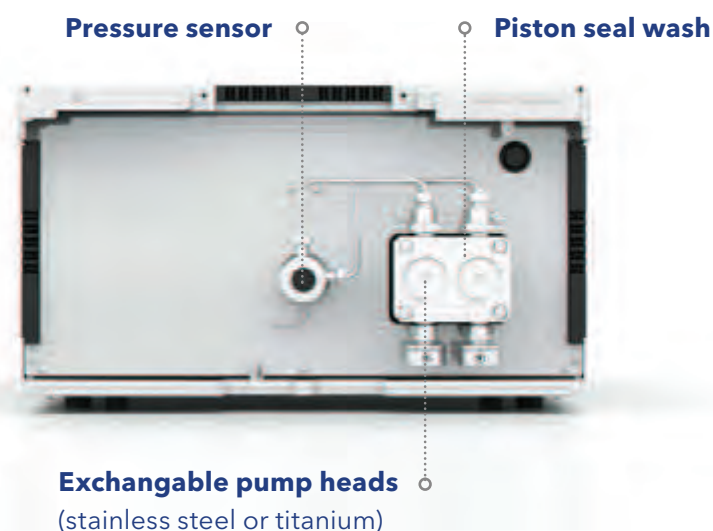
Eluent delivery

Precise and reliable pumps covering a wide flow range for various gradient and solvent selection options.

AZURA® Pump P 2.1L

The preparative HPLC pump AZURA P 2.1L covers a wide flow rate and pressure range. It has been designed for the purification of milligram to gram samples. The integrated automatic RFID pump head recognition allows a quick adaptation to various applications.

- **Flow rate** up to 1000 ml/min
- **LPG** and **HPG** gradient options
- Supports **constant pressure mode**



Gradient options of Pump P 2.1L

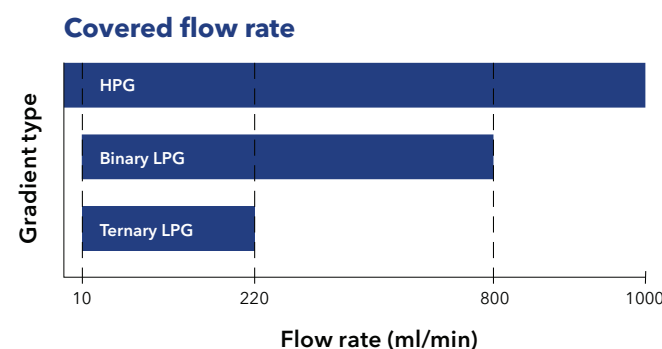
A **low pressure gradient (LPG)** module dynamically composes the eluent on the inlet-side or low pressure side of the pump head, by quickly switching between the different solvent channels. We offer binary or ternary LPG upgrade modules for the isocratic P 2.1L.

The eluent in a binary **high pressure gradient (HPG)** system is composed by combining the solvent flows of two isocratic pumps.

Pump head	Max. pressure	Best working conditions
100 ml	400 bar	1 - 80 ml/min
250 ml	200 bar	2.5 - 200 ml/min
500 ml	100 bar	5 - 400 ml/min
1000 ml	50 bar	10 - 800 ml/min

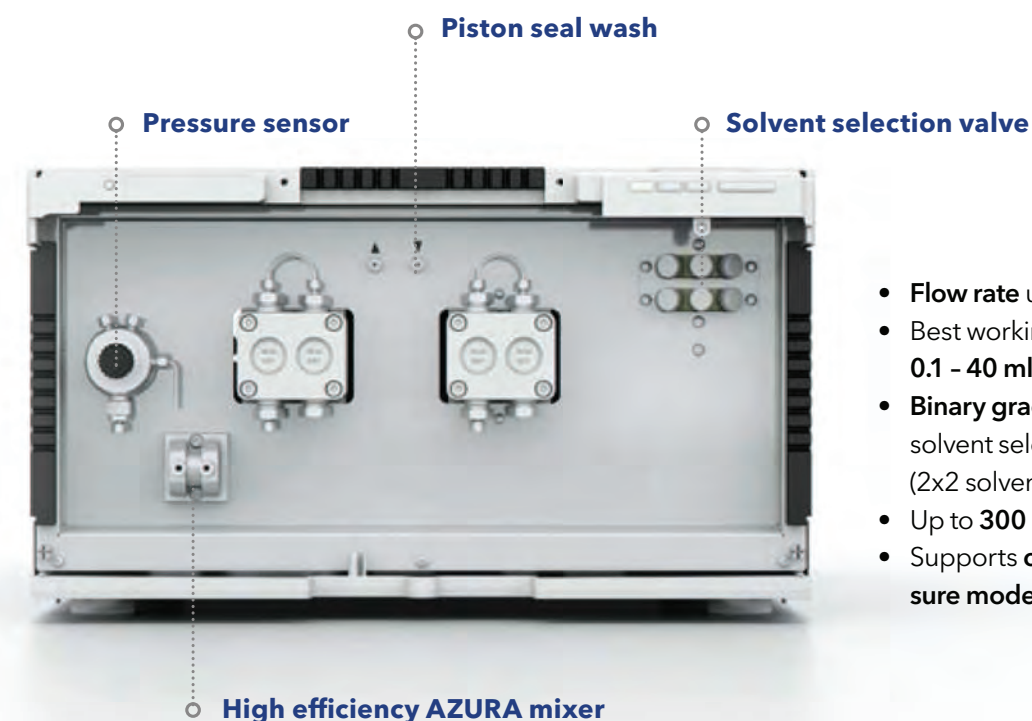


Binary LPG valve block for the pump head



AZURA® Pump P 6.1L

The AZURA semi-preparative pump P 6.1L with 50 ml pump head is available as an isocratic or binary HPG pump. It is made for medium-size purification tasks and upscaling processes.

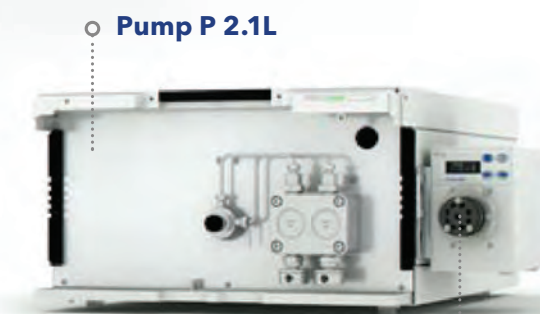


- **Flow rate** up to 50 ml/min
- Best working conditions: **0.1 - 40 ml/min**
- **Binary gradient** with solvent selection valve (2x2 solvents)
- Up to **300 bar**
- Supports **constant pressure mode**

Solvent selection

For automated solvent change, a solvent selection valve can be attached to the pump P 2.1L.

For semi-preparative purification tasks, the pump P 6.1L features a built-in 2x2 solvent selection valve (high pressure gradient version).



8 Port solvent selection valve (PEEK) (stainless steel coming soon)

AZURA® Assistant ASM 2.1L

A flexible combination module

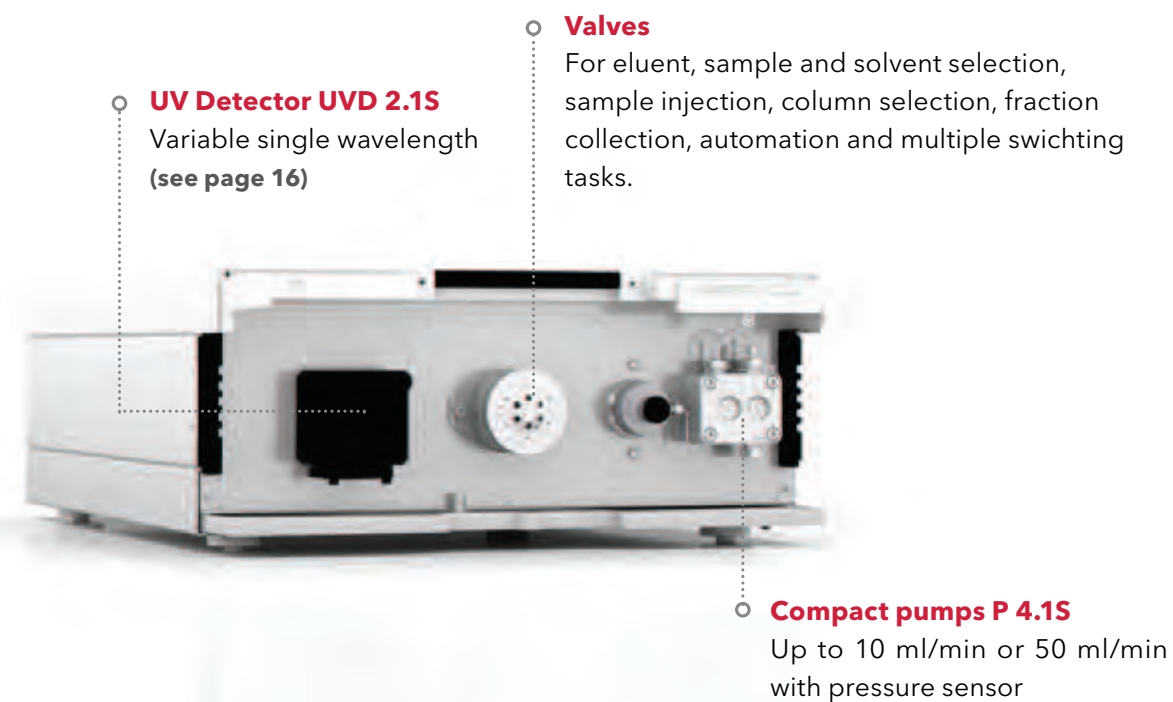
The assistant ASM 2.1L is a compact combination module which can be equipped with up to three device modules. Available for selection are valves, pumps and an UV detector. An assistant can be the basis of a whole HPLC system including a pump, valve and a detector, just like the AZURA compact prep system (page 30). As part of a larger system, the ASM 2.1L is extremely versatile.

Depending on the integrated modules, the assistant fulfills many different tasks such as sample and solvent selection, sample injection, column switching, fraction collection, eluent delivery or UV detection.

The concept of the flexible combination of device modules combines the highest functionality with minimum space requirements.

Configure your assistant

Can be equipped with combinations: valves, pumps, and one detector



Sample injection

Adapt the sample injection mode to your preparative task.

Injection valve

The simplest way to inject your sample into the system. Use a manual injection valve and choose from a large range of different sample loops.



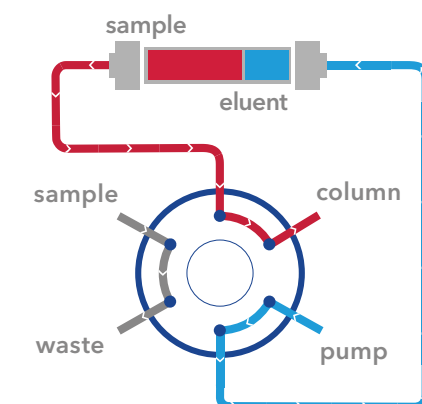
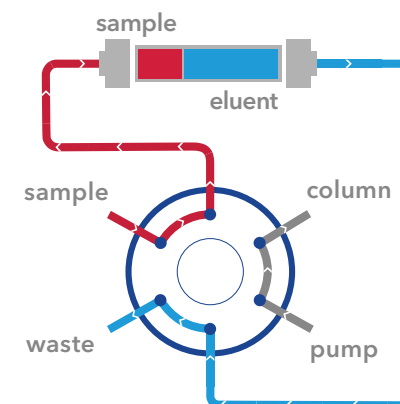
KNAUER offers several injection valves for 1/16" and 1/8" tubing. The wetted parts are made of stainless steel or PEEK to cover a broad range of applications. Injection can be done either manually via hand lever or automated with a valve drive.



VariLoop for sample injection

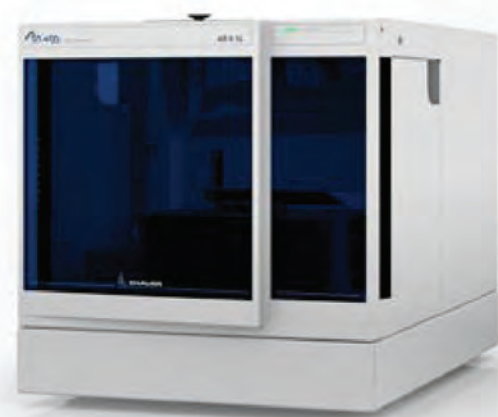
The KNAUER VariLoops are the perfect solution for the injection of medium up to high sample volumes (up to 40 ml). The sample loop can be emptied completely or partially as well as filled completely

or partially. This allows you to work very flexible and easily switch between different sample sizes while keeping constant and reproducible injection volumes for every sample size.



Autosampler AS 6.1L

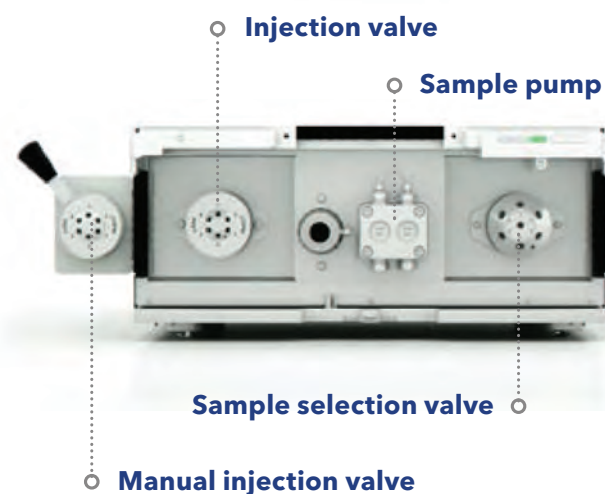
Sample injection can be easily automated with an autosampler. The AS 6.1L can inject up to 10 ml per injection. Sample tray temperature control from 4 - 40°C is optional available. It can handle either 30 samples in 10 ml vials or up to 768 samples in well plates.



Sample Injection Assistant ASM 2.1L

The AZURA sample injection assistant for preparative LC is based on the multifunctional AZURA element ASM 2.1L. It is designed to automate injection of larger sample volumes and features a sample selection valve, a sample pump, and an injection valve.

Simply attach your sample vessels via 1/8" tubing to the multiposition valve and automate injection with the integrated sample pump and injection valve.



Sample pump

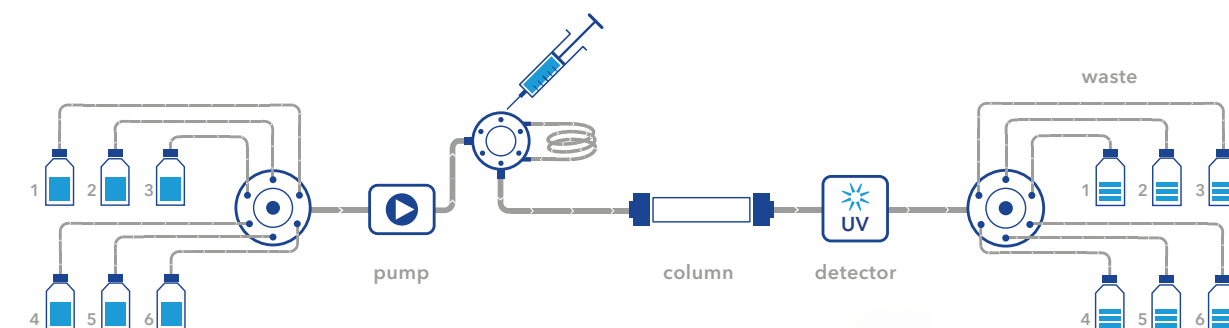
Standalone or integrated in an assistant module ASM 2.1L: The compact pump AZURA P4.1S is perfect for feed injection.

- 10 and 50 ml **exchangeable pump head**
- **Flow rate** range:
0.01 - 50 ml/min (50 ml pump head)
0.001 - 10 ml/min (10 ml pump head)
- Pump heads available in **stainless steel** or **ceramics**
- Best working conditions:
1 - 40 ml/min (50 ml pump head)
0.1 - 8 ml/min (10 ml pump head)



Multiposition valves for automation

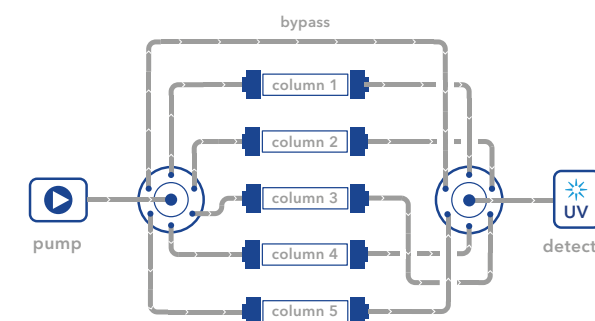
Eluent selection and fractionation



When automated selection of eluents is required, up to 12 different eluents can be attached to the preparative system. Multiposition valves fulfill many different tasks: solvent and sample selection, fractionation and column switching.



Multiposition valve

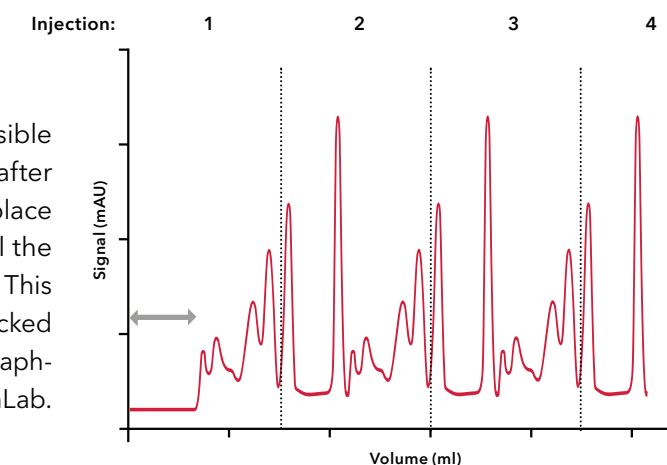


Column switching

Switching valves are ideal for screening and scale-up. They can be easily integrated into your system at pressures up to 400 bar and maximum flow rates of 300 ml/min.

Stacked injection

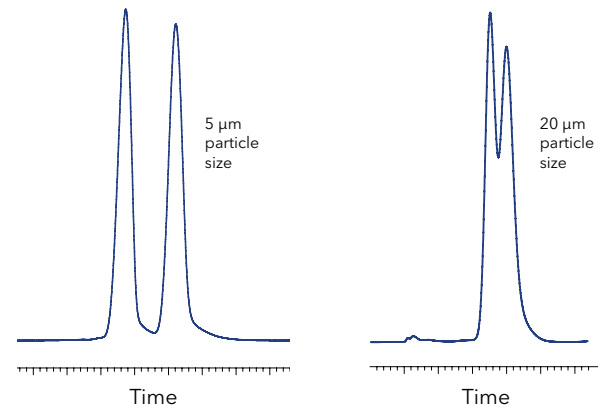
With the stacked injection function, it is possible to perform different runs automatically one after the other. The injection of the next run takes place during the current run, so that the time until the elution of the first peak can be fully exploited. This increases efficiency, saves time and eluent. Stacked injection can be operated with the chromatographic data systems (CDS) PurityChrom® and OpenLab.



Peak and solvent recycling

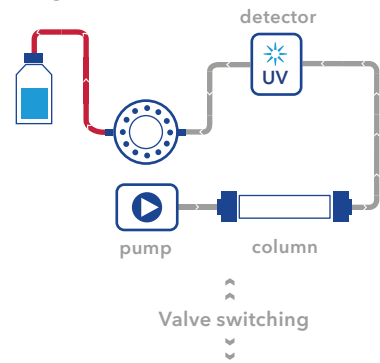
An example

A separation can be much more demanding after upscaling from analytical to preparative scale. In many cases a baseline separation is not possible anymore, so time and money consuming method development or hardware adjustments are necessary. The AZURA Prep LC system is well-suited to apply the peak recycling technique to solve demanding resolution tasks. Additionally, solvent recycling can be applied to save eluent, if it can be considered clean.

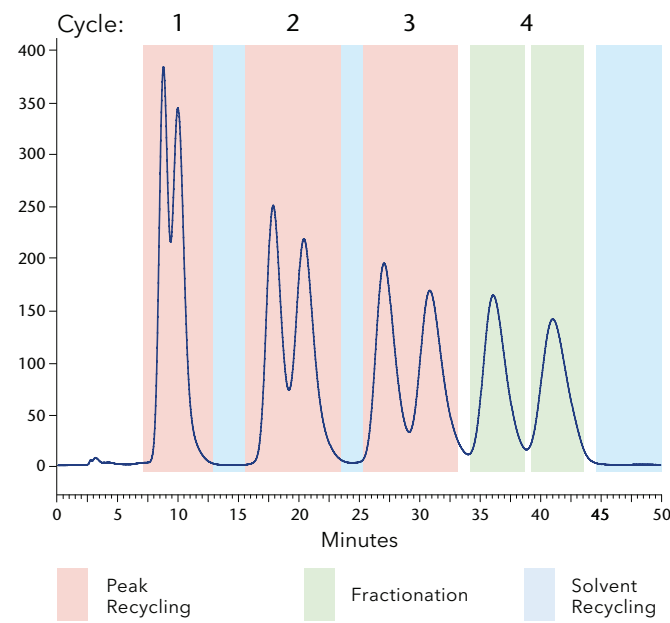
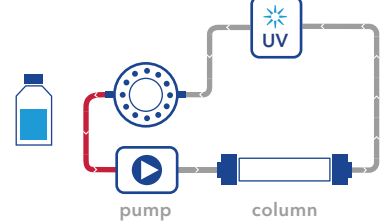


Comparison of analytical and preparative chromatogram.

Collecting mode



Recycling mode

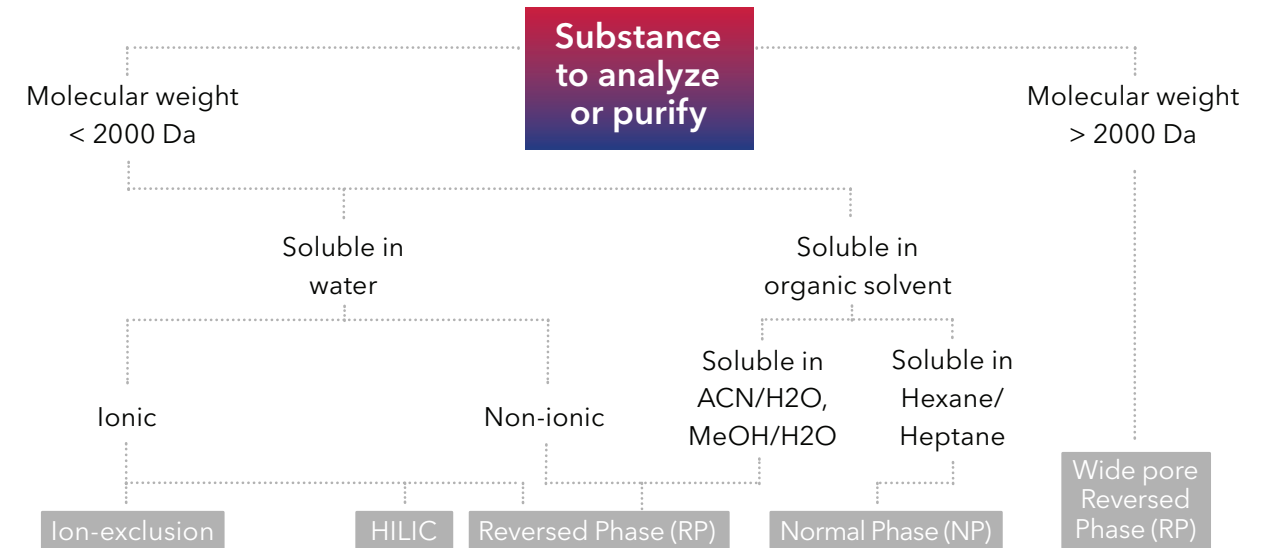


Successful peak separation with recycling mode.

KNAUER preparative columns

Find the perfect column from the large KNAUER portfolio

This flow chart gives you a guideline how to select the right column for your application. Start at the top and follow the decision lines all the way down to find a column recommendation.



H-form (USP L17)	Silica (USP L3)	Classical C18 (USP L1)	Silica (USP L3)	Wide pore classical C18 (USP L1)
Ca-form (USP L19)	NH2 (USP L8)	Hydrophilic/ aqueous C18 (USP L1)	NH2 (USP L8)	Wide pore C18A hydrophilic/ aqueous (USP L1)
Pb-form (USP L34)	Zwitter-ionic HILIC (USP -)	Hydrophobic/ pH stable C18 (USP L1)	Diol (USP L20)	Wide pore C8 (USP L7)
Na-form (USP -)		Classical C8 (USP L7)	Cyano (USP L10)	Wide pore C4 (USP L26)
		Hydrophilic/ aqueous C8 (USP L7)		
		Hydrophobic/ pH stable C8 (USP L7)		
		C4 (USP L26)		
		CN (USP L10)		
		Phenyl (USP L11)		
Eurokat		KNAUER Eurospher Eurospher II		KNAUER Eurosil Bioselect

Detection

KNAUER gives you the opportunity to analyze nearly every compound due to a large portfolio of HPLC detectors. For the achievement of your analysis goals and for matching your separation scale, our detectors are flexible in the setup, including flow cells and fiber optics. Our product line of UV/VIS detectors ranges from single variable wavelength to 8-channel diode array detectors with 3D scan capability.



Detector	UVD 2.1S	UVD 2.1L	MWD 2.1L	DAD 2.1L	DAD 6.1L
	Compact and versatile UV detector	Reliable UV/VIS detector for a wide spectrum of applications	Robust multi-channel UV/VIS detector	Versatility through a wide flow cell range	High-end diode array detector with outstanding performance
Wavelength	190-500 nm	190-750 nm	190-700 nm	190-700 nm	190-1000 nm
Channels	1	1	8	8	8
3D scan				•	•
Fiber optics available	•	•	•	•	•

Flow cells for UV/VIS and DAD detectors

Select from an impressive range of easily exchangeable preparative and semi-preparative flow cells for UV/VIS and DAD detectors. With capillary connections ranging from 1/16" to 1/4" and TRI-Clamp adaptations, optional fiber optics technology and a variety of flow cell wetted materials, a wide spectrum of applications can be covered.

Max. flow rate	Connectors	Path length	Volume	Max. pressure	Fiber optics available
50 ml/min	1/16"	3 mm	2 µl	300 bar	•
250 ml/min	1/16"	0.5 mm	3 µl	200 bar	•
1000 ml/min	1/8"	0.5/1.25/2 mm	1.7/4.3/6.8 µl	200 bar	•
10000 ml/min	1/4"	0.5/1.25/2 mm	1.7/4.3/6.8 µl	200 bar	•

Fiber optics technology

More flexibility

Fiber optic cables offer the possibility to separate the flow cell from the detector. This enables demanding applications such as measuring directly after a heated LC column or in hazardous environments, allowing safe operation of the instrument while maintaining performance.

Safe operation

When working at high flow rates, separation of the flow cell and the detector is a safety feature. In case of leakages, no damage to the detector occurs. Fiber optics are available in a customized length of up to 10 meters.



○ Flow cell with 1/4" TRI-Clamp connection

AZURA® RID 2.1L HighFlow Preparative refractive index detector

The AZURA RID 2.1L HighFlow is a sensitive and competitively priced differential refractometer. It is suitable for detecting compounds with little or no UV activity such as alcohols, sugars, lipids or polymers in high concentrations. This instrument is designed for use in semi-preparative and preparative HPLC for flow rates up to 100 ml/min. Optional are higher flow rates possible with a flow splitter. The intelligent temperature control guarantees fast baseline stabilization and stable operation.



Special detection

Choice of specialized detection technology, fully integrated in PurityChrom®. Suitable for preparative LC with the help of a flowsplitter.

Light Scattering Detector Sedex LC

Sensitive universal detection with the possibility to run gradients

As a universal detector, an ELSD detector offers numerous possibilities for detecting substances that have few or no chromophores. Since the eluents are evaporated, the use of non-UV-compatible solvents poses no problems and the ELSD is gradient compatible.

Target analytes: Carbohydrates and similar compounds, detergents, ionic and non-ionics, artificial sweeteners, antioxidants, amino acids, lipids, peptides, polymers, pestizides, proteins, steroids.



AZURA® Conductivity Monitor CM 2.1S

The Conductivity Monitor CM 2.1S can monitor salt gradients with flow rates of up to 100 ml/min and a maximum pressure of 100 bar. It supports a wide measurement range of 0.01 mS/cm - 999 mS/cm. Flow cells in PEEK for both analytical and preparative scale are available.



○ pH option available

Mass spectrometry solution by KNAUER

4000 MiD

The KNAUER 4000 MiD is a single quadrupole MS with a spraychip ionization source. It is able to perform both positive and negative electrospray ionization and features the scan modes full scan, SIM and interleaved. With its integrated oil-free pump, it achieves a mass accuracy of +/- 0.3 m/z when performing a full scan. With a mass resolution of 0.7 m/z (FWHM), it is the perfect choice for mass directed purification.



All-in-one solution

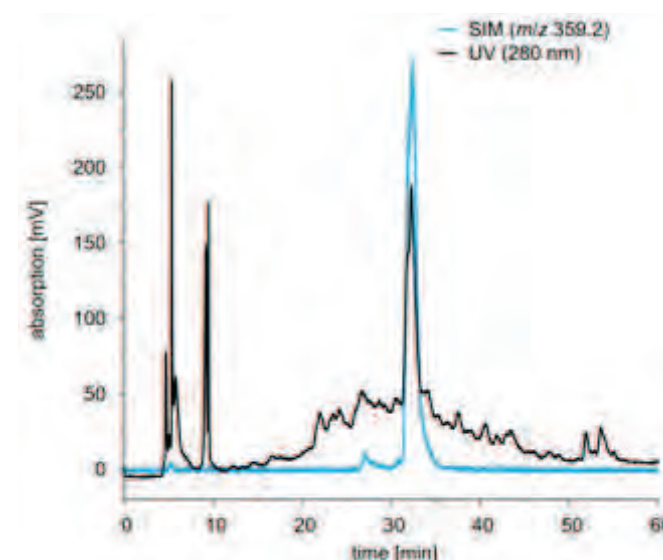
With the integrated vacuum system and integrated electronics inside of one box the KNAUER 4000 MiD brings mass spectrometry to places where no other spectrometer can be deployed.

Target analytes

With a mass range of 800 m/z the KNAUER 4000 MiD can be used for a broad variety of applications. In combination with the KNAUER MiDas it is the ideal choice for preparative chromatography and direct introduction methods.

Easy to use

With the KNAUER 4000 MiD and its simple 'plug and play' consumables mass spectrometry gets as easy as possible.



Purification of a natural product by mass directed fractionation

Black: UV trace at 280 nm
Blue: MS trace (SIM) for target compound

Fraction collection

Collect large quantities or large numbers of fractions

KNAUER offers different valves for fraction collection and variations of trusted fraction collectors. Whether you are doing research and development or production, there is an appropriate solution that suits your application.

Fractionation modes:

- Manually** - collection by direct control
- Time-based** - collection at defined time points
- Peak-based** - collection according to detector signal
- Threshold function** - collection according to any signal

Fraction collectors

LABOCOL Vario 4000 / Plus

The LABOCOL Vario 4000 fraction collectors are characterized by their high robustness and optimal ratio of dimensions/benefit. The user is not limited to given rack types. The rack layout can be designed according to individual needs. Free rack design. Any rack type can be integrated by de-

fining the number of fraction vessels and their position. The wide application area makes the Vario 4000 series ideal for use in research and development as well as in production. The Vario 4000 models differ in the base area and the flow rate range.



Rack type
80 Tubes 18 mm
125 Tubes 10.5 mm
20 Tubes 36 mm
39 Tubes 26 mm
24 Centrifuge tubes 50 ml

Foxy® R1 and R2

The Foxy® R1 fraction collector can be adapted to a broad spectrum of applications. Flow rates of up to 125 ml/min for Foxy R1 and 1000 ml for Foxy R2 are possible. Fractions can be collected into 96 well microplates, standard tube sizes, bot-

tles and many more. For essentially unlimited volumes, funnel racks can direct fluids to any collection vessel or downstream process. Both devices can be operated stand-alone or in the chromatography software PurityChrom®.

Rack type
144 Vials 12 mm
144 Vials 13 mm
100 Vials 16 mm
36 Vials 25 mm
2 Microwell plates 96
60 Tubes 1.5 ml
72 Centrifuge tubes 15 ml
36 Centrifuge tubes 50 ml
2 x 9 Bottles 480 ml*
36 Funnels with vinyl tubing
26 Funnels with vinyl tubing*



* Foxy R2 only

Fractionation valves



8 Port Multiposition valve
for 1/8", PEEK
7 fractions + waste
(stainless steel coming soon)

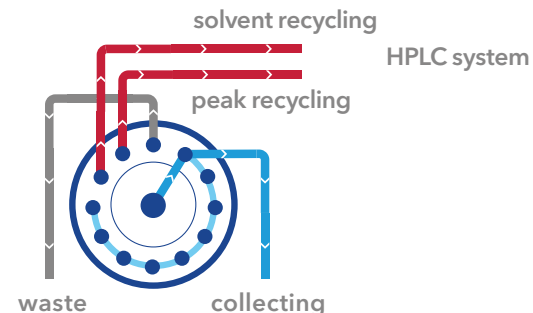
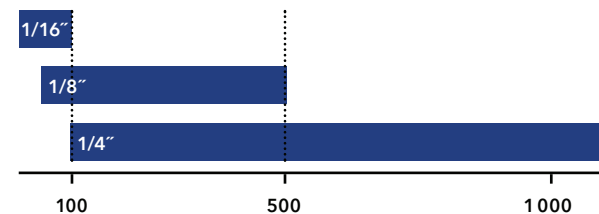


12 Port Multiposition valve
for 1/8", SST
11 fractions + waste



16 Port Multiposition valve
for 1/16", SST
15 fractions + waste

Fractionation valves max. flow rate (in ml / min)



Device	Max. flow rate (ml/min) 1/16"; 1/8"; 1/4"	Racks	Different rack types	Max. fractions 1/16"; 1/8"; 1/4"
Valve	100 / 500 / 1000			16 / 12 / 10
Foxy R1	25 / 125 / -	1	15	up to 144
Foxy R2	- / 125 / 1000	2	15	up to 288
Labocol Vario 4000	100 / 500 / 1000	3	5**	72*
Labocol Vario 4000 Plus	100 / 500 / 1000	5	5**	120*

* For 50ml tubes

** Device supports other racks via user-defined position setting.

Temperature control

Increase performance. Minimize solvent viscosity.

Eluent and Column Heater

When performing preparative LC at temperatures above 40°C in air-conditioned laboratories, a uniform temperature distribution is essential. With the Eluent Heater, solvent temperature can be precisely controlled using the integrated touchscreen. It supports flow rates of up to 500 ml/min and is cleanroom compatible.



Column Heating Sleeve

Our column heating sleeves are the perfect solution for thermostating your preparative column hardware. Available for all preparative KNAUER column dimensions at temperatures up to 100 °C. Custom dimensions, clean room compatible and autoclavable materials are available on request.



Column Oven

This oven can heat up to 120 °C. It can accommodate up to 8 KNAUER columns with max. 250 x 50 mm inner dimensions.








Pump Head Heater

Electrical heating element for pump heads. Temperature can be controlled using the eluent heater or a single device control unit.



Accessories

Improve system performance, organize your lab bench, and work more conveniently with the right accessories.

Accessory	Features	Benefit
Pump head inlet 	<ul style="list-style-type: none"> Connect one 1/4" tube to the AZURA Pump P2.1L Adapters for other diameters available 	For high flow rates and viscous eluent
Mass flow controller 	<ul style="list-style-type: none"> Unmatched accuracy at flow rates up to 833 ml/min Compatible with PurityChrom® 	Precisely monitor the eluent flow
Dynamic Mixing Chamber 	<ul style="list-style-type: none"> Effective homogenization of eluents 	Better performance
VariLoop 	<ul style="list-style-type: none"> Variable injection volume and multiple injections 	Adapt the sample volume to your application
Interface Box IFU 2.1 LAN 	<ul style="list-style-type: none"> Highly precise analog data acquisition 4-channel input/output Sample rates of up to 50 Hz (one channel only) 	Add any detector with analog output to your system

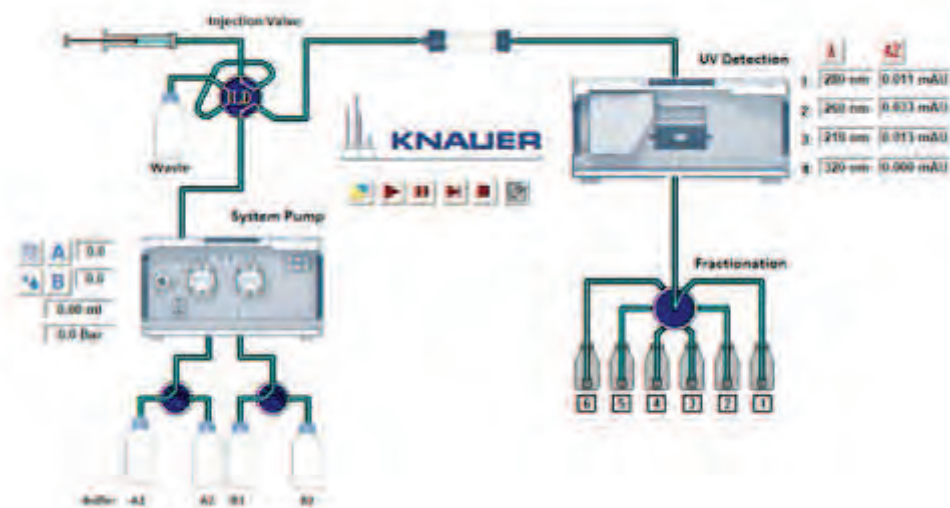
Accessory	Features	Benefit
Column Base 	<ul style="list-style-type: none"> Holds up to three preparative columns 	Flexible operation with up to three columns
Benchtop Rack 	<ul style="list-style-type: none"> Install AZURA systems at space-limited sites, especially in cold rooms. 	Space-saving solution for AZURA system setup
Air Sensor 	<ul style="list-style-type: none"> Detect end of buffer or end of sample with PurityChrom® Up to four air sensors per system For transparent tubings with 1/16" or 1/8" or 1/4" outer diameter 	Protect column from air damage and support automation (e.g. sample injection)
AZURA® Click 	<ul style="list-style-type: none"> Attach air sensor, pressure control, AZURA Organizer or your interface box to the side panel of your AZURA L device 	Organize your system.
AZURA® Organizer 	<ul style="list-style-type: none"> Attach columns from 5 mm to 26 mm diameter, falcon tubes, pH flow cell 	Organize accessories directly at the system and reduce dead volume
Flow Splitter 	<ul style="list-style-type: none"> Adjustable valve for precise direct control over split ratios Ultra low dead volume fluidic design 	Collect fractions while using your preferred detection method

Software solutions

PurityChrom®

PurityChrom is a powerful software to control your preparative system. Get familiar with PurityChrom in shortest time and with no effort due to the intuitive and clearly structured user interface. Choose a time- or volume based workflow by just

clicking one button. Create methods with highest flexibility to realize complex application without losing easy handling. Offline licenses for creating methods and data evaluation are for free. The software is 21 CFR part 11 compliant.



Intuitive Control

PurityChrom includes intuitive data evaluation with peak recognition and integration. Due to its high flexibility, methods can be developed according to specific demands. You have the option to create a method based on volume, column volume, or time. There is also the possibility to pause your method during a run. The hold function provides you with complete control over your chromatography process. Solvent visualization calculates the consumption of solvent for the current run and prevents your column from running dry.

System visualization

The system visualization offers a graphical representation and allows easy handling even of complex flow processes. Furthermore, each device which is displayed in the fluidic scheme can be manually controlled, giving the opportunity to optimize, change and adapt your conditions during the run.

ClarityChrom® CDS

ClarityChrom is an easy-to-use chromatography data system (CDS) for workstations. Besides support of all KNAUER devices, components and systems from more than 45 manufacturers are also supported. ClarityChrom® includes the drivers for several fraction collectors and supports peak recognition by level and/or slope. The manual fraction control and the option to use the KNAUER electric valves for fractionation give you even more flexibility.

- Fraction collecting via peak recognition (level only, slope only, level AND / OR slope - incl. self-learning) or single event (unconditional, timed event)
- Easy to collect: waste, collect to position / collect to next, solvent recycling
- Direct control during a run - manually switch to: collect, waste, solvent recycling
- Consecutive runs: easily find your chromatogram by clicking on your fraction

OpenLab

OpenLAB CDS EZChrom Edition provides support of devices from KNAUER and many other manufacturers. The KNAUER fraction collector control option includes the drivers of several fraction collectors and supports fractionation by time, the peak recognition by level and/or slope, also with spectral confirmation. Collect Slices allows for setting a desired volume for each fraction, within the defined fraction vial volume. The manual fraction control and the option to use the KNAUER electric valves for fractionation gives you more flexibility. The combination of virtual detector and virtual fraction collector allows for optimizing the fractionation settings from an existing chromatogram of your separations without any physically existing device and, therefore, without the loss of solvent or target substance.

Mobile Control (Chrom)

The hand-held Mobile Control (Chrom) allows a complete overview of all devices of the AZURA systems on one screen. Remotely check important parameters or control and monitor devices. The touch-optimized user interface facilitates navigation using just your fingers. The display software Mobile Control provides full access to AZURA devices. Change device settings, set operating parameters, automate device control or check the system status and GLP data... Mobile Control features all functionalities of a device display.

Do you want to acquire data without the overhead of a chromatographic data system? Mobile Control Chrom features data acquisition from AZURA detectors in addition to full device control. For simple applications Mobile Control Chrom might be the appropriate software solution - basic, easy-to-use and cost-effective!

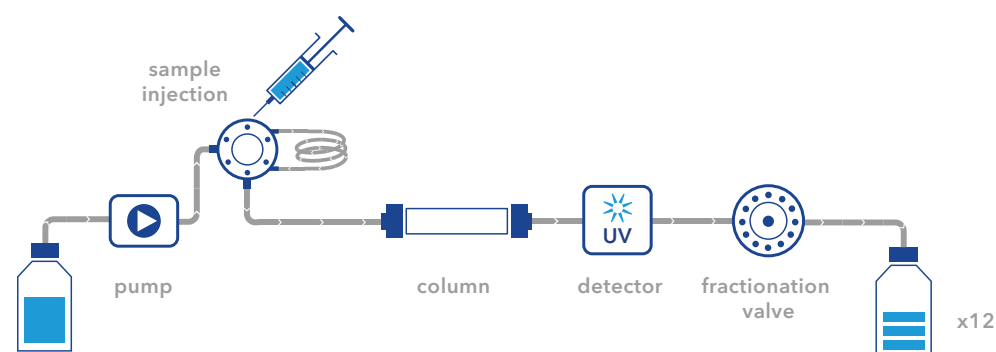
Chromeleon™ 7

Chromeleon is one of the most wide-spread chromatography data systems. It offers a broad range of third-party drivers and can be easily used with existing HPLC systems. Chromeleon drivers for many KNAUER devices are available.



AZURA® Compact Prep HPLC System

The AZURA® Prep Compact system is the perfect start into to preparative chromatography. With the complete, semi-preparative HPLC system you master your isocratic purification tasks.



Compact Prep System

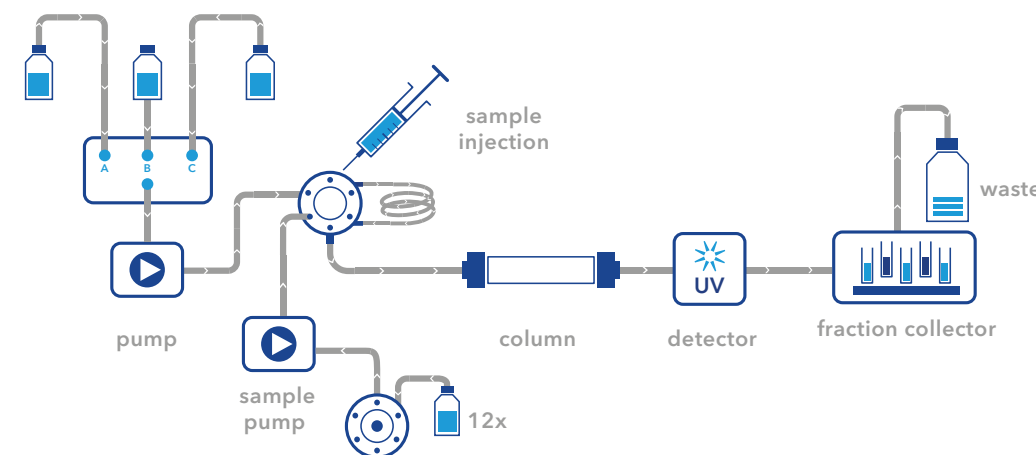
One manual injection can purify several hundred milligrams at up to 50 ml/min. Detection takes place via a versatile UV/VIS detector. The intuitive preparative software PurityChrom controls

the compact system and regulates the fraction collection via a 12-port fractionating valve. Thanks to its compact design, the AZURA Prep Compact system finds its place in every laboratory.

- Complete **semi-preparative** isocratic HPLC system with **low space** requirements
- Injection valve incl. **500 µl sample loop**
- **UV/VIS detector** with one variable wavelength
- Intuitive **PurityChrom®** software
- **Compact** and **expandable**



Easy upgrade without big investment



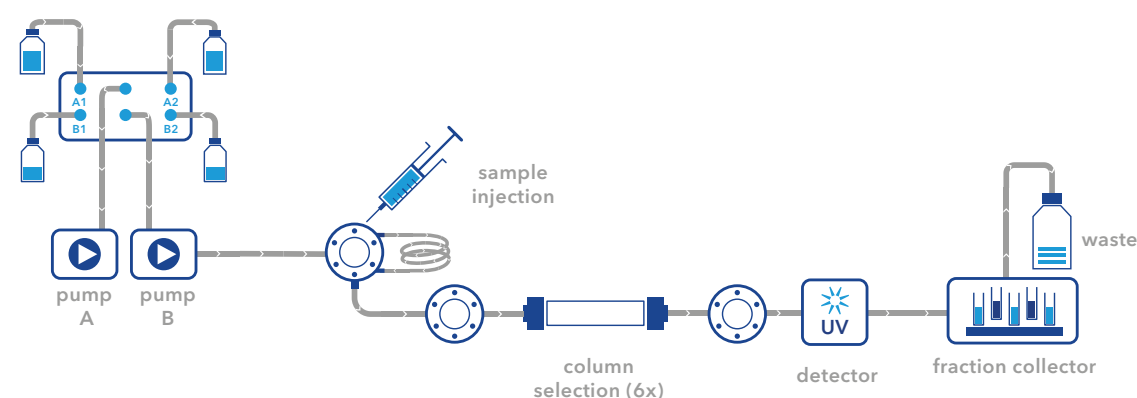
Pilot Prep System

After starting preparative chromatography with the space-saving prep system, the requirements for your purification tasks can quickly increase. The existing Compact System (50 ml/min) can be expanded to a Pilot System (220 ml/min) by investing in a fraction collector and a preparative pump. All components of the Compact System are fully integrated into the Pilot System.

- Pilot Ternary gradient HPLC system
- Injection valve incl. **500 µl sample loop**
- **Sample pump with automatic sample selection**
- **UV/VIS detector** with one variable wavelength
- Intuitive **PurityChrom®** software
- **Fraction collector**

AZURA® Lab Prep HPLC System

The Lab Prep LC system is designed for your more demanding semi-preparative separations. You can customize a highly flexible LC system with the freely combinable components. With a maximum flow rate of 50 ml/min it is possible to separate up to several hundred milligrams per run.



- Lab Prep HPLC system with **binary high pressure gradient**
- **Column selection**
- Injection valve incl. **500 µl sample loop**
- **UV/VIS detector** with one variable wavelength
- Intuitive **PurityChrom®** software
- **Fraction collector**



Method transfer from analysis of chamazulen to preparative scale

Chamomile plants are known for their medical properties, having among others anti-inflammatory, analgesic and sedative effects. These are due to the various phenolic compounds, one of them matricine is converted during the distillation process to chamazulene. The characteristic blue color of chamomile essential oils as "chamomile blue" is due to chamazulene. It has anti-inflammatory and anti-oxidant activity. The present application tested preparative HPLC to purify chamazulene from commercially available "chamomile blue" oil.

Results

Fractionation/Purification

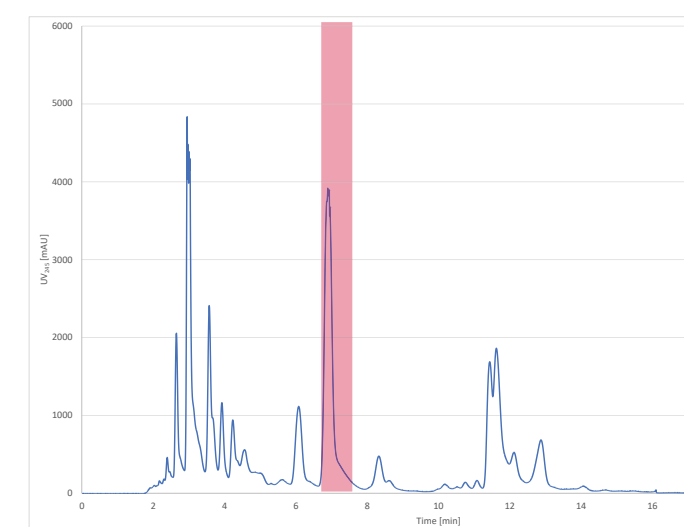


Fig. 1: Chromatogram of preparative separation of chamazulene blue, collected fraction highlighted in red, 1 mL sample injection

Fraction analysis

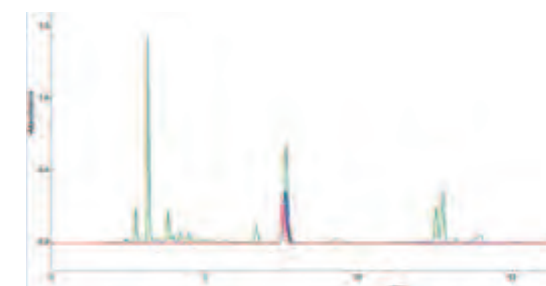


Fig. 2: Chromatogram overlay

The collected fraction was analysed by analytical HPLC and revealed nearly 100 % purity. Chromatogram overlay of the fraction, chamazulene standard and the sample clearly showed the successful purification of chamazulene (Fig. 2).

Further the comparison of the fraction spectra (Fig. 3) and chamazulene spectra (Fig. 4) revealed that the purified fraction was chamazulene.

The separation of chamazulene was optimized in analytical scale and the two step gradient method transferred to preparative scale. Chamazulene purification was performed on C18 250x20 mm column, 25 ml/min. Fractionation of chamazulene was conducted by threshold function of PurityChrom software.

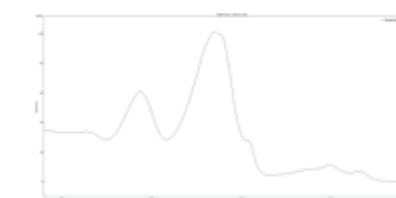


Fig. 3: Spectral view fraction

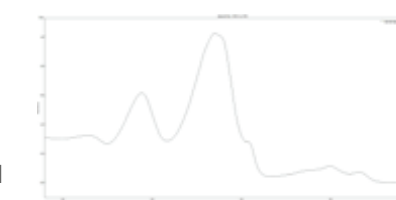
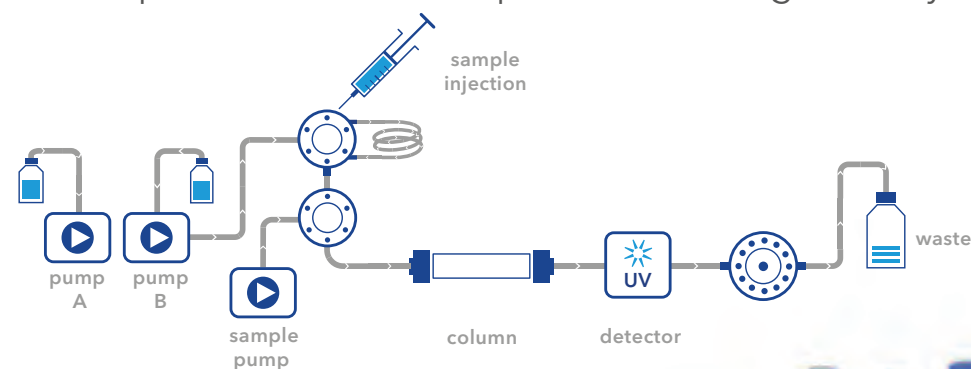


Fig. 4: Spectral view chamazulene standard

AZURA® Pilot Prep HPLC System

Choose the Pilot Prep LC system if you want to increase your productivity even more. As for the AZURA Lab Prep LC system you can freely build up your system. Flow rates up to 1000 ml/min and loads up to several grams are possible. Optional peak and solvent recycling can be set up to increase separation power and reduce separation costs significantly.



- Pilot Prep HPLC system with **binary high pressure gradient**
- **Sample pump**
- Injection valve
- **UV/VIS detector** with one variable wavelength
- **12 Port** fractionation valve
- Intuitive **PurityChrom®** software

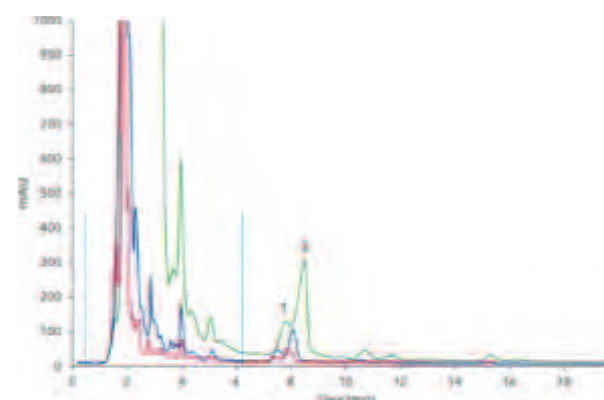


Improved purity by combining online SPE with preparative LC

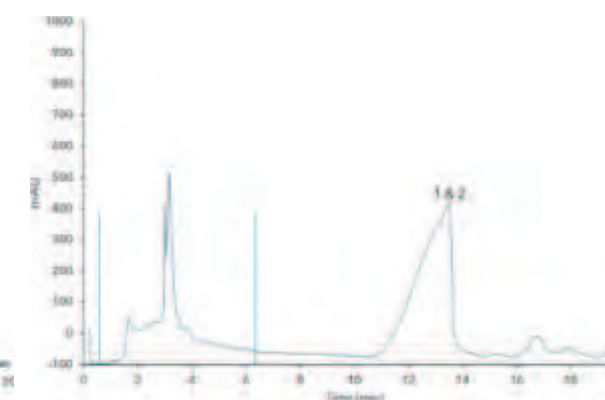
Steviol glycosides are the main sweetening compounds in *Stevia rebaudiana* and are often used as natural sugar substitutes. To enable a commercial usage, the plant extracts need to be purified. In this work preparative online SPE (solid phase extraction) with the AZURA Pilot Prep LC was investigated for improvement of overall purity due to reduction of matrix contamination.

The steviol glycoside rebaudioside A is the main compound of interest as it is the sweetest and less bitter compound of the extract. Often *Stevia* products contain a mixture of rebaudioside A and stevioside. The development of a purification method with high yield of rebaudioside A, only few stevioside impurities, and high throughput increases the economic output of *Stevia* production.

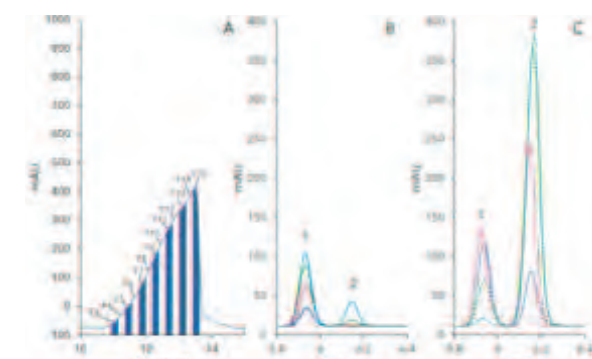
Results



Overload experiments on preparative column, 200 µL (red), 500 µL (blue), 2000 µL (green); 1) rebaudioside A, 2) stevioside, blue bars - matrix, 25°C, 22 ml/min



Preparative online SPE, 10 mL loading; 1) rebaudioside A, 2) stevioside, blue bars - matrix, 25°C, 22 ml/min

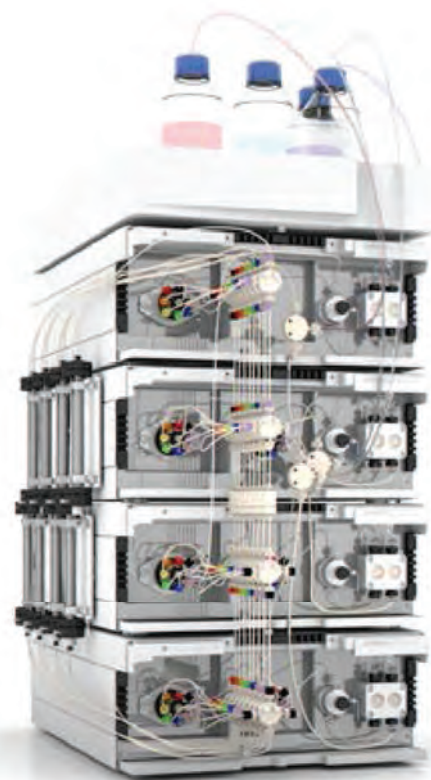


Fraction analysis of preparative online-SPE purification (Fig.2) of rebaudioside A (1) and stevioside (2); a) F3 (blue), F4 (red), F5 (green), F6 (light blue); b) F7 (red dashed), F10 (blue dashed), F12 (green sashed), F15 (light blue dashed); c) fractionation of target peak, 5 mL fractions

Fig. 1 shows the batch LC without online SPE. The matrix peak (1-5min) negatively affect the separation abilities. In comparison Fig 2 shows that the automated SPE process significantly decreased the matrix. The fraction analysis revealed that only a small part of the overlapping peak contained nearly pure rebaudioside A; fractions 3-5 approx. 15 mL with >90 % rebaudioside A and <10% stevioside (Fig. 3, B). The later fractions contained high amounts of stevioside but also still rebaudioside A (Fig. 3 C). The results showed that purification of highly pure rebaudioside A is possible by an additional online-SPE .

For more information visit www.knauer.net (Application Note VFD0171)

AZURA® SMB systems



Simulated moving bed chromatography (SMBC) is increasingly applied as a separation technique in the pharmaceutical industry, production of fine chemicals and in the field of bioengineering. SMB is a method in process chromatography that enables substance mixtures to be continuously separated and extracted in two fractions. By repeated use of the SMB process each partial fraction can be separated into a further fraction - down to binary substance mixtures.

Typically, the SMB process is set up in advance for a two component mixture. Following this, both substances can be immediately extracted in pure form.



For more information about SMB:
www.knauer.net/smb

What is the difference between batch LC and SMBC?

Batch chromatography (single-column)	SMB chromatography (multi-column)
Unlimited number of fractions	Two fractions, no waste
Recovery typically below 80%	Recovery up to 100%
EITHER high purity OR high yield	High purity AND high yield
Isocratic or gradient	Isocratic
High solvent consumption	Can be as low as 10% of batch consumption
Very diluted product	Product concentration comparable with input concentration (feed)

Lab & pilot scale

Meet your continuous separation task in lab and pilot scale with a biocompatible or a stainless steel version.

Operation modes

Besides the standard SMB configuration, different zone configurations and Open/-Closed-Loop are possible to optimise your process.

Intuitive software

Based on our preparative software you adapt easily to our continuous chromatography software.

About KNAUER

Based in Berlin, KNAUER is a medium-sized, owner-managed company that has been serving the sciences since 1962. We develop and manufacture scientific instruments of superior quality for liquid chromatography. The range includes sys-

tems and components for analytical HPLC / UHPLC, preparative HPLC, fast protein liquid chromatography (FPLC), multi-column chromatography / simulated moving bed (SMB), and osmometry.



KNAUER Academy



Worldwide partner in science since 1962

We separate molecules and unite people.



CEO and owner Alexandra Knauer

The founder Dr. Herbert Knauer and his wife Roswitha are still active as advisers in the company to this day. The couple's daughter, Alexandra Knauer, has been managing director

and owner of the company since 2000. Several awards for outstanding products and innovations as well as entrepreneurial excellence make KNAUER a „leading employer“.

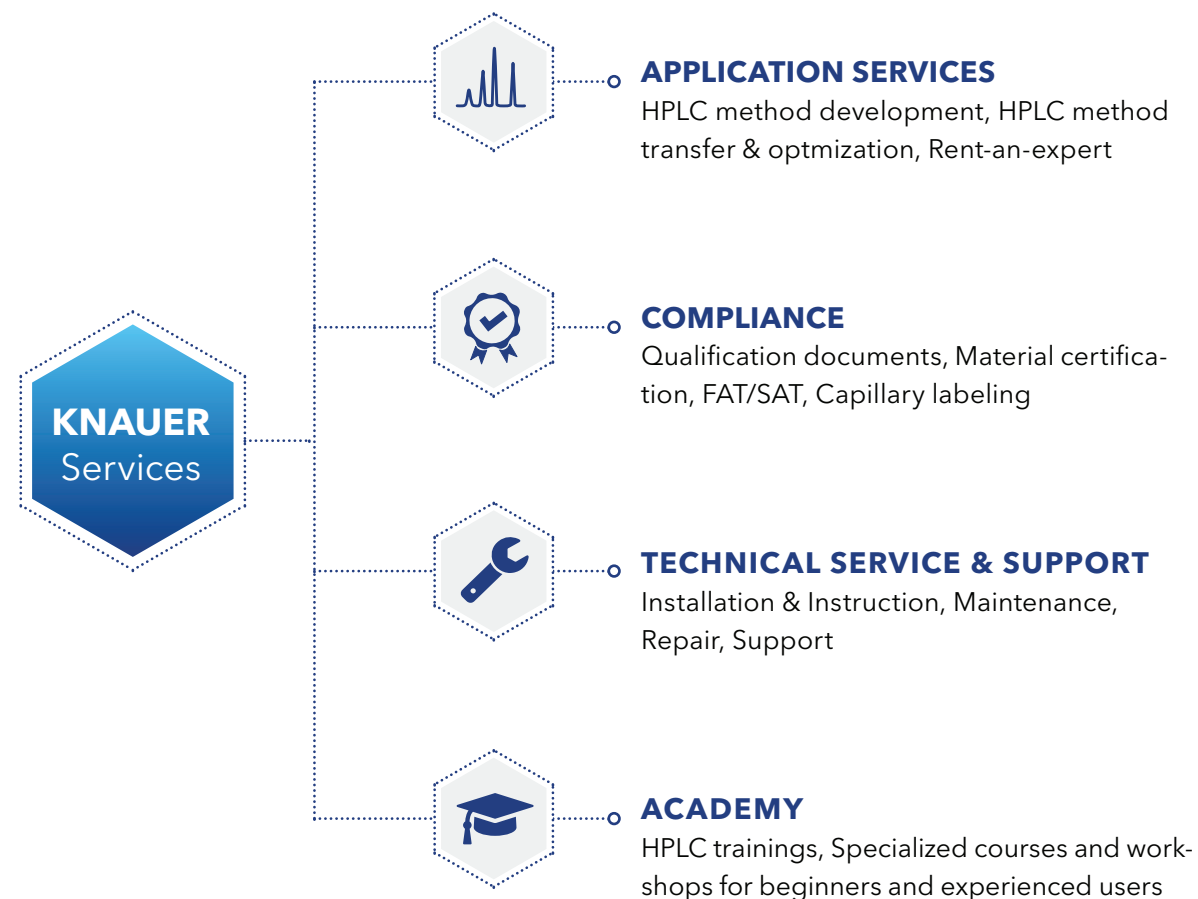
Independent and family owned



www.knauer.net



KNAUER Services



Contact us

All standard user instructions, helpful video tutorials, and a structured section of frequently asked questions is freely accessible on our web page www.knauer.net.

If you need further support, our friendly Support team is happy to help you via e-mail, phone or Team Viewer. They will work with you personally until all issues are resolved.

Phone: +49 30 809727-111 (workdays 9-17h CET)

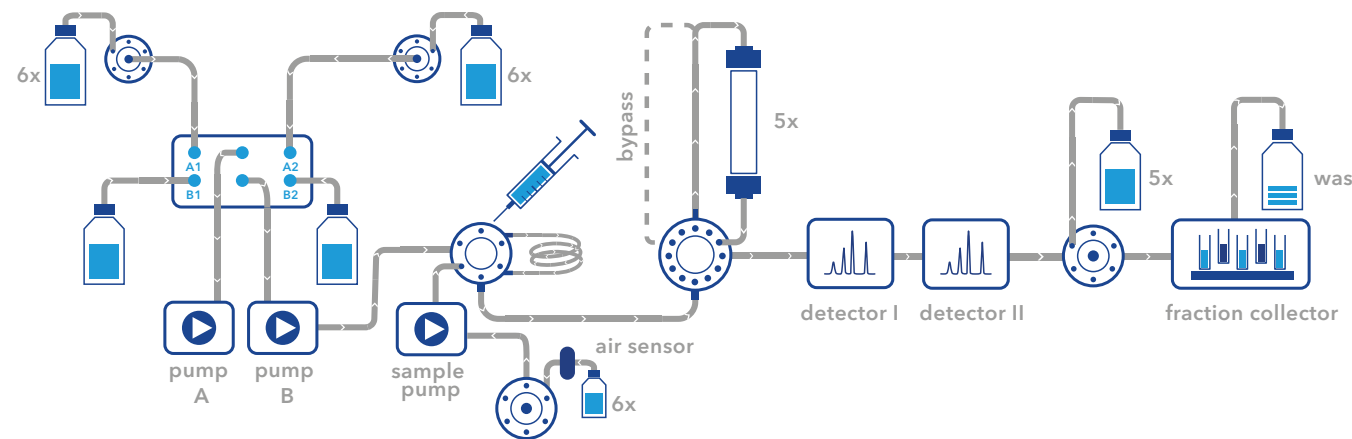
Email: support@knauer.net

System configurator

Preparative HPLC by KNAUER

MAKE YOUR PRESELECTION

Stainless steel Titanium



SOLVENT SELECTION & DELIVERY

- 50 ml/min binary gradient pump P 6.1L
- x 100 ml/min pump P 2.1L
- x 250 ml/min pump P 2.1L
- x 500 ml/min pump P 2.1L
- x 1000 ml/min pump P 2.1L
- Ternary gradient module for pump P 2.1L
- Binary gradient module for pump P 2.1L
- x solvent selection valve

SAMPLE INJECTION

- Injection valve
- Sample pump module
- Sample selection valve: x inlets
- Autosampler AS 6.1L

COLUMN SELECTION & THERMOSTAT

- Column selection (two columns or one bypass)
- Column selection high flow (5 columns, one bypass)

DETECTION

- UV/VIS single wavelength
- UV/VIS multiwave length
- DAD 2.1L
- Conductivity
- pH
- Refractive index
- Light Scattering
- 4000 MiD
- A/D-converter (integration of further detectors)

FRACTION COLLECTION

- Fractionation valve
- Foxy fraction collector with fixed rack types
- Labocol fraction collector with individual rack types
- Rack for fraction collector
- Flow splitter

ACCESSORIES

- x Airsensor main pump
- x Airsensor feed pump
- Mass flow controller
- AZURA Click
- AZURA Organizer
- x Tubing 1/16"
- x Tubing 1/8"
- x Tubing 1/4"
- Workstation (Windows)

SOFTWARE

- ClarityChrom®
- OpenLAB®
- PurityChrom®
- Chromeleon™
- Mobile Control

COMMON APPLICATIONS

- Reversed phase
- Normal phase
- other...
- System Qualification

Science Together



AZURA® Bio purification

Extensive and flexible FPLC solutions

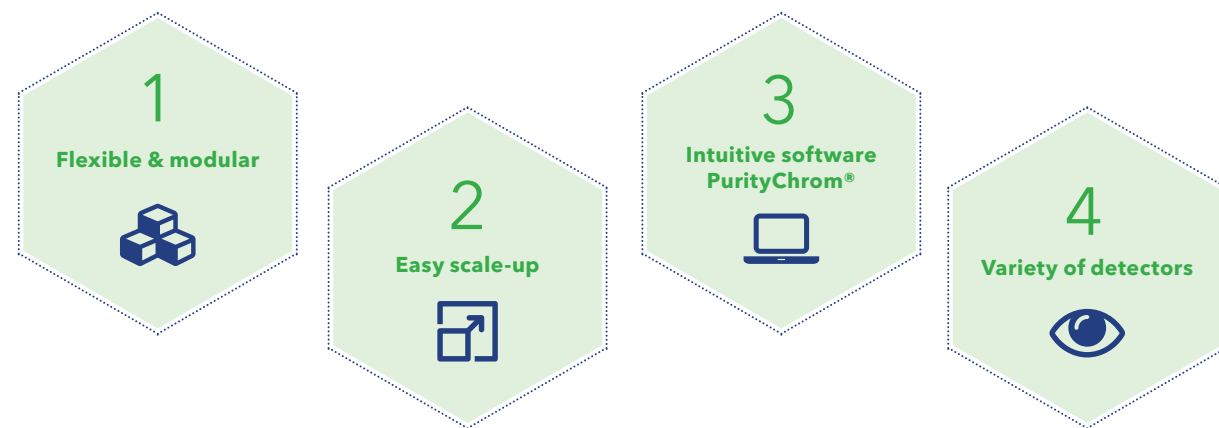


KNAUER protein purification

The flexible FPLC platform

AZURA® Bio purification systems

Complete solutions for FPLC on a minimum footprint: AZURA FPLC systems combine flexibility and reliability. The biocompatible/metal-free FPLC is the perfect choice for your protein purification task.



Design your AZURA Bio purification system to your needs. Multiple functionalities such as automatic sample injection via autosampler, column switching, buffer and sample selection as well as fraction collection enable the user to automate the purification process.

A large range of different detectors make your target molecules visible. Different flow rates and compatibility to columns from all vendors offer maximum flexibility. The intuitive software PurityChrom® combines all the advantages of a versatile purification software.

Fast Protein Liquid Chromatography (FPLC)

FPLC is a liquid chromatographic method for purification of large biomolecules like proteins. External factors like high temperature, high pressure, extreme pH, or solvents can disturb the protein structure and are therefore avoided in FPLC. Be-

sides, the method uses column materials out of agarose or polymer material which are very sensitive against pressure fluctuations and air bubbles. **We designed our systems to meet your purification challenges!**

AZURA® Bio purification: You choose the method

Size Exclusion Chromatography (SEC)	Affinity Chromatography (AC)	Ion-Exchange Chromatography (IEX)	Hydrophobic Interaction Chromatography (HIC)
Separate according to size. See page 22 for a specialized AZURA system for SEC.	Specific binding of protein of interest. See page 23 for a specialized AZURA system for AC.	Separation takes place according to the charge of the protein and gradient elution.	Separation is performed based on hydrophobic interaction and gradient elution.

Purification strategy: Often a sequence of different methods is used in purification.



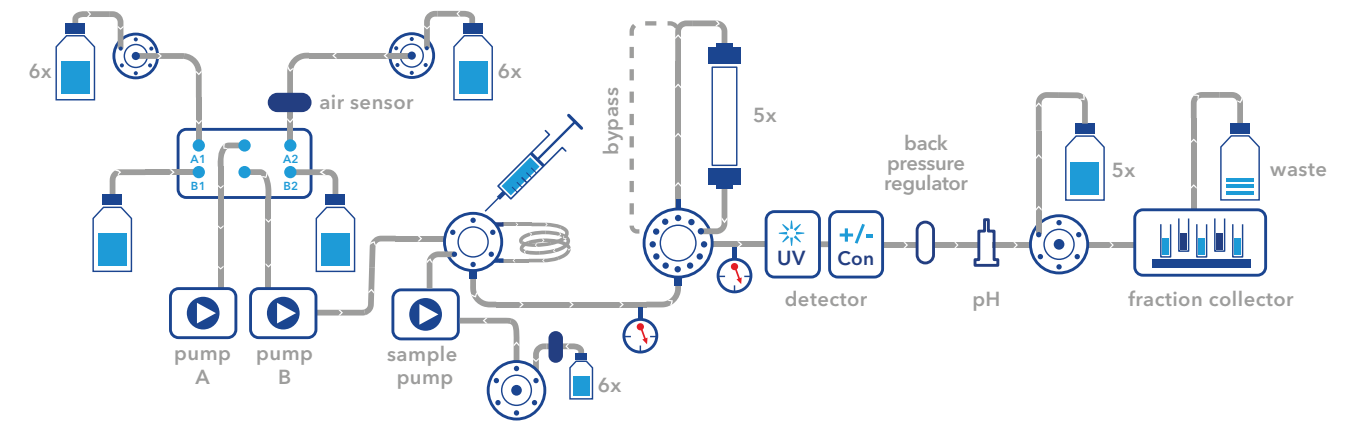
Normally a combination of methods is used in protein purification.

- The "capture" step purifies the protein from the crude extract.
- The "intermediate" step removes further contamination.
- The aim of the final "polishing" step is to get rid of all remaining impurities in order to gain a highly purified product.
- Automatization of two purification steps is possible using the especially designed AZURA Two step purification system (see page 24).

AZURA® Bio Lab purification system

From simple to complex, from lab to pilot scale: Design your AZURA® FPLC system according to your purification task!

AZURA Bio Lab allows you to create FPLC systems with highest independence. Just pick your modules and build-up the system yourself. Continue flexibility with intuitive PurityChrom® software.



BUFFER SELECTION & DELIVERY

SAMPLE INJECTION

COLUMN SELECTION

DETECTION

FRACTION COLLECTION

All common FPLC methods are supported.

Detection ○ Various detectors: UV/VIS, diode array, refractive index, fluorescence and a selection of flow cells (see page 16)

PurityChrom® ○ Intuitive and high flexible software (see page 26)

All columns are supported.

Column selection valves (page 11)

Sepapure® FPLC columns (page 12)

Automate your purification (page 20)

Cold-room operation is supported.

Injection ○ Manual or automated injection: using a valve, feed pump or autosampler valves for sample selection available (for a maximum of 8 samples)

Buffer delivery ○ Quaternary, binary pumps with flow rates up to 10 ml/min or 50 ml/min (see page 6)

Buffer selection ○ Integrated buffer selection valve for 4 buffers, extra buffer selection valves available (see page 6)

Fraction collection ○ Various fraction collectors and fractionation valve and a selection of racks for 96-well-plates up to several liters (see page 18)

Conductivity ○ With pH option (see page 16)

Scale-up from lab to pilot

Choose the Pilot series if you want to increase your productivity even more. Upscale our Lab configuration with same flexibility, software PurityChrom® but minimal footprint. Just transfer and upscale your methods. Flow rates up to 1000 ml/min and loads up to several grams are possible. Find more information: www.knauer.net

Configure your AZURA Bio purification system
Find all FPLC products on the following pages.

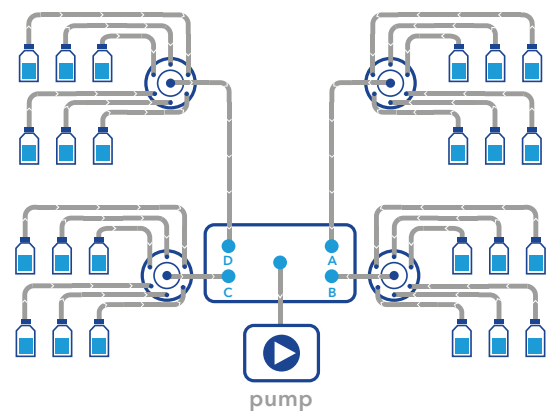
Buffer delivery

Precise and reliable pumps covering a wide flow rate range, gradient and buffer selection options.

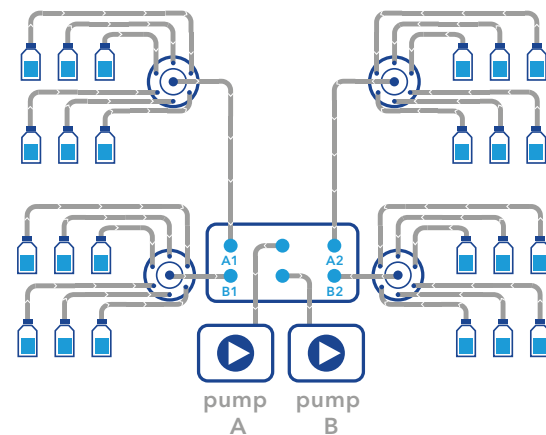
Buffer selection

Automated switching between buffers is important for method development, column cleaning and regeneration. The pump P 6.1L features a build-in 2 x 2 buffer selection valve (A1, A2 and B1, B2) or 4 x buffer selection valve (A, B, C, D).

You can extend buffer selection with additional valves each for up to 8 buffers.



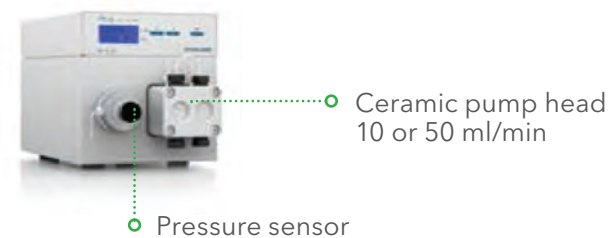
AZURA pump P 6.1L LPG - Quaternary gradient



AZURA pump P 6.1L HPG - Binary gradient

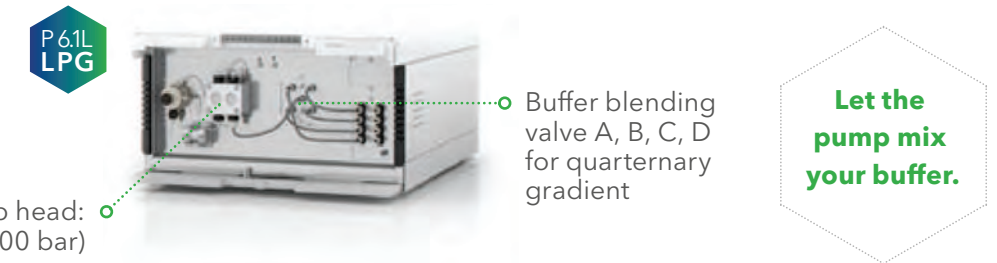
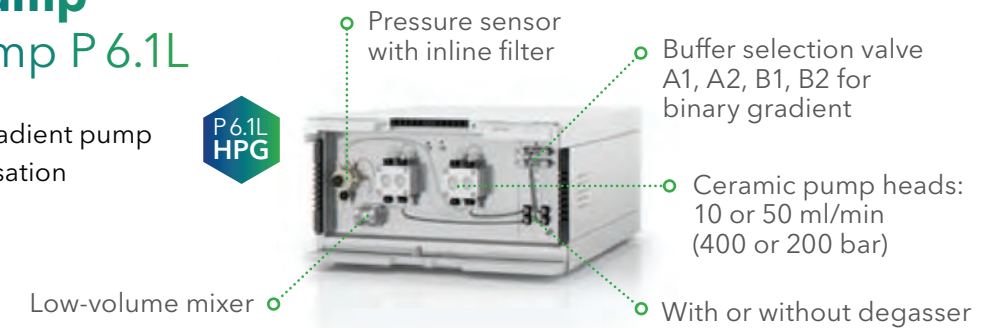
Compact pump AZURA® Pump P 4.1S

Isocratic pump with small footprint for dedicated applications or sample loading.



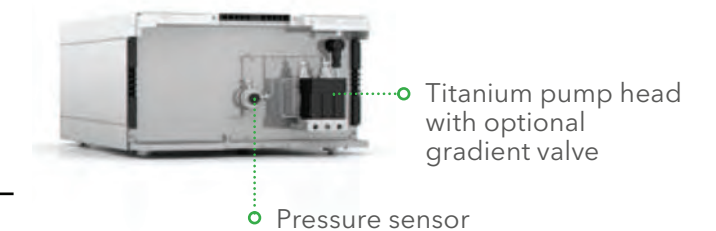
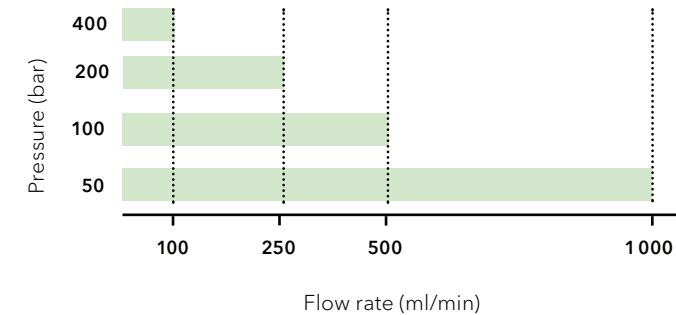
Gradient pump AZURA® Pump P 6.1L

High-performance gradient pump optimized for low pulsation



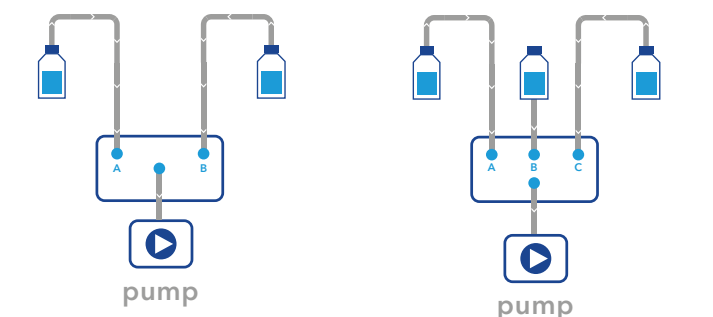
Scale-up pump AZURA® Pump P 2.1L

Pumps for high flow rates



Gradient options

From binary to quaternary gradient, with additional P 2.1L pumps or cost-effective binary low pressure gradient (2 x 1 buffers, up to 800 ml/min) or ternary low pressure gradient (3 x 1 buffers, up to 220 ml/min).

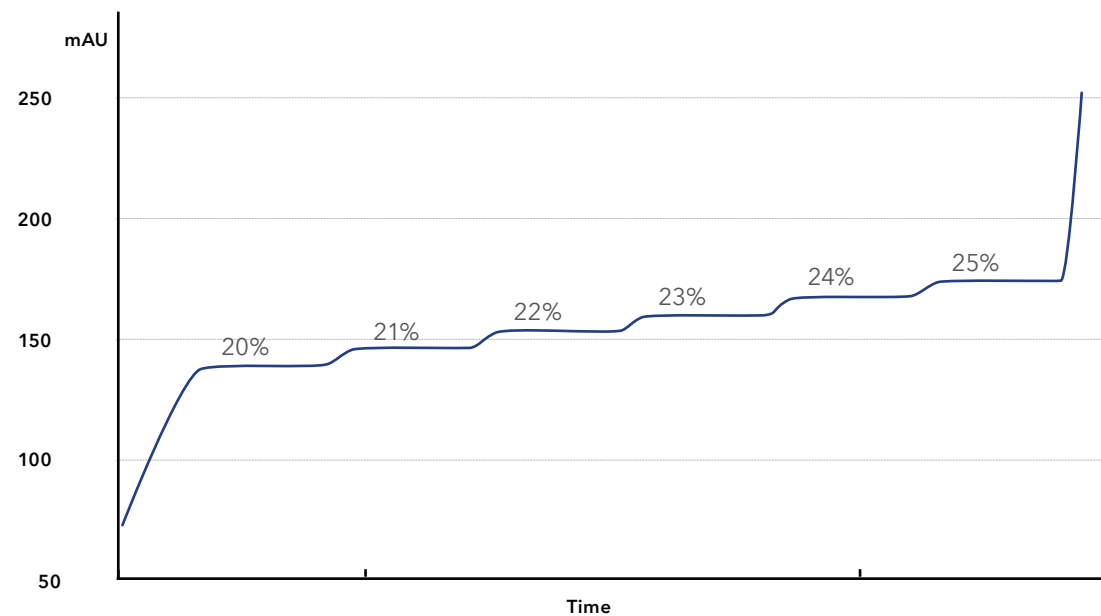
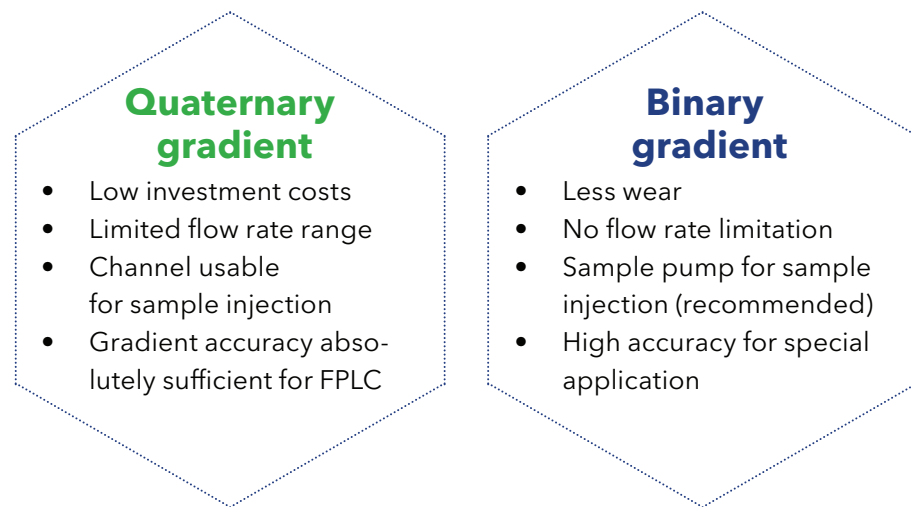


Binary low-pressure gradient Ternary low-pressure gradient

Binary or quaternary gradient?

A quaternary low pressure gradient (LPG) module* dynamically composes the buffer on the inlet-side or low pressure side of the pump head, by quickly switching the selection valve between the dif-

ferent channels. The buffer in a binary high pressure gradient (HPG) system is composed by combining the flow of two pumps.



Excellent gradient reproducibility of 0.3 % RSD, overlay of 6 repetitions at 1 ml/min run with pump P 6.1L low pressure gradient version

*For higher flow rates available as ternary or binary gradient (see page 7).

AZURA® ASM 2.1L Assistant

A flexible combination module

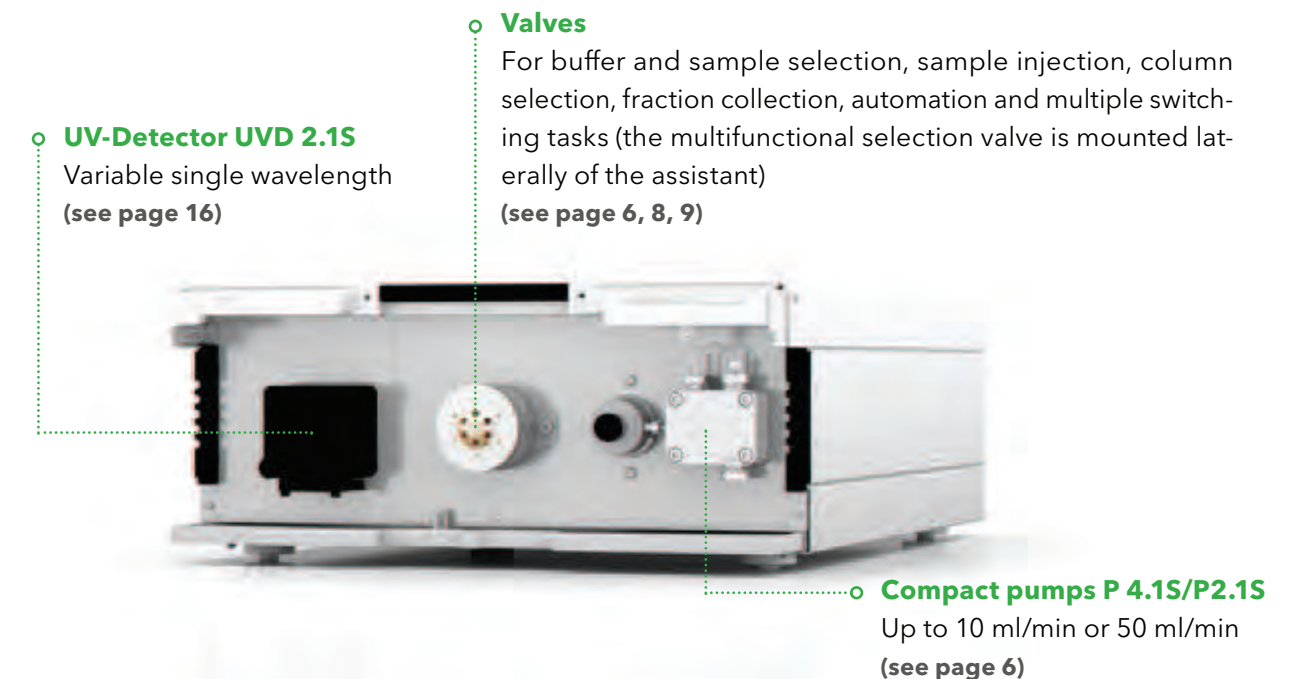
The assistant ASM 2.1L is a compact combination module which can be equipped with up to three device modules. Available for selection are valves, pumps, and a UV detector. An assistant including a pump, valve, and detector features a compact FPLC system, like AZURA Bio SEC or AZURA Bio AC. As a part of a larger system, the ASM 2.1L is extremely versatile. Depending on the integrated

modules the assistant fulfills many different tasks like sample and buffer selection, sample injection, column switching, fraction collection, buffer delivery or UV-detection.

The concept of the flexible combination of device modules combines the highest functionality with minimal space requirements.

Configure your assistant

Can be equipped with combinations: valves, pumps, and one detector



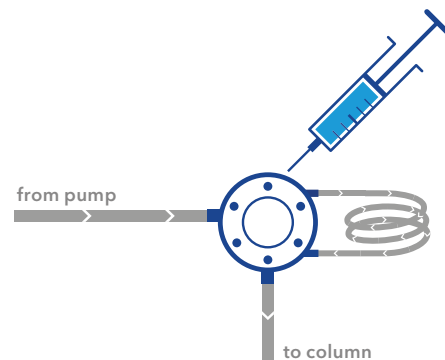
For detailed information on device modules and assistant configuration: www.knauer.net

Sample injection

Choose between manual or automated sample injection. Available modules include injection valve, sample pump, or autosampler.

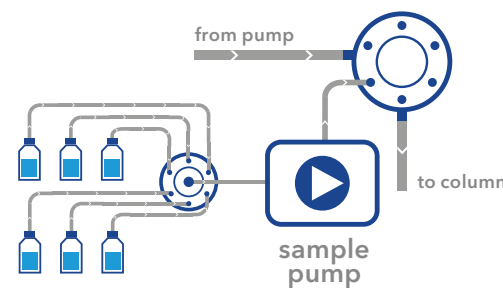
Injection valve

Integrated into assistant or standalone module: The AZURA 2-positions valve is perfect for injection of small sample volumes. Connect 1/16" tubings for flowrates up to 100 ml/min. For higher flowrates use the injection valve for 1/8" tubing. Various sample loops are available.



Sample pump

Integrated into assistant or standalone module: The AZURA P 4.1S is perfect for injection of larger sample volumes. Repetitive sample injections by using the pump for automated sample loop filling.



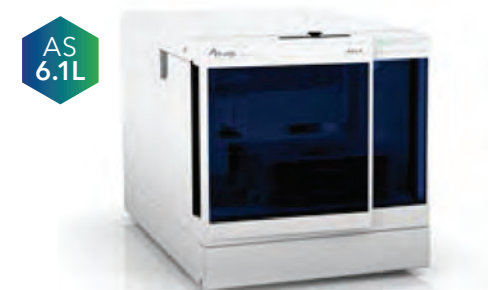
Do you have many samples?

You can extend your configuration with additional valves each for up to 8 samples.

Autosampler

Process many different samples fully automatically with the Autosampler AS 6.1L.

- Up to 10 ml injection volume
- From microtiter plates to standard vials
- Active cooling
- Fully supported by PurityChrom® software
- Metal-free

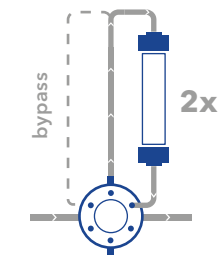


Column selection

Different options for column selection are available.

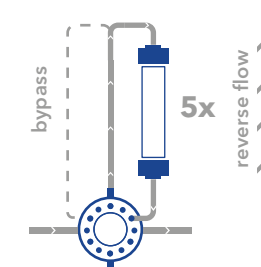
2-position valve

- Select two columns or one column and one bypass
- Flow rates up to 500 ml/min possible



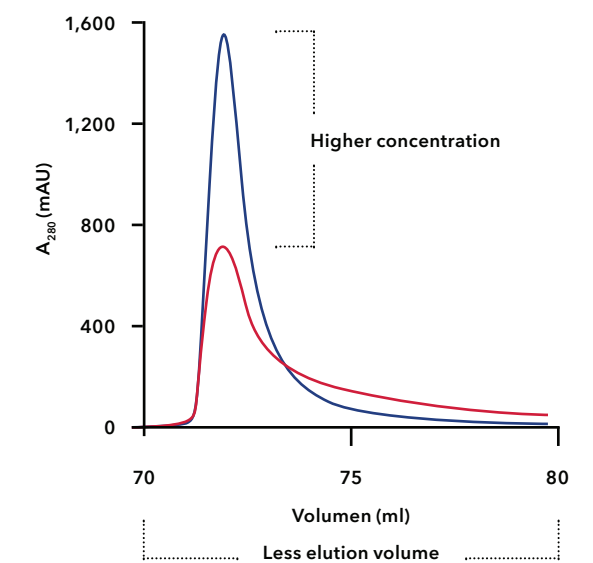
Multifunctional selection valve

- For up to 5 columns and 1 bypass
- Reverse flow
- Flow rates up to 50 ml/min



Why is the reversed flow option popular in affinity chromatography?

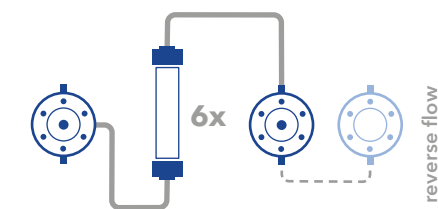
In affinity chromatography your target molecules will accumulate at the top of the column. Elution in the same direction dilutes your target molecule along the column. By elution with reversed flow you increase the concentration while decreasing the sample volume.



The option has two major advantages. Clean your columns more efficiently using reverse flow. By this you elute contamination the shortest way and minimize damage to the column.

Higher flow rates?

Use the column selection assistant to select six columns assuring a flow rate up to 500 ml/min. An additional valve allows to reverse the flow.



Sepapure®

Bio purification columns and media

The perfect addition to any FPLC system

Size Exclusion Chromatography (SEC)

In size exclusion chromatography biomolecules are separated according to their size. There are two different methods used in SEC which are defined by the matrix of the FPLC columns.

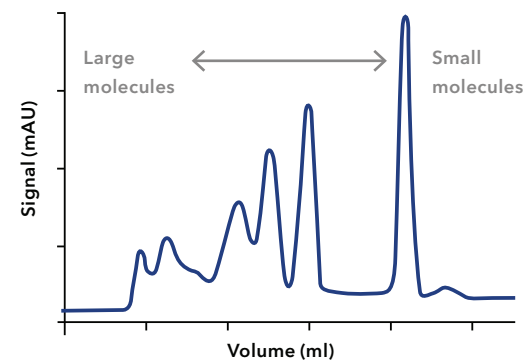
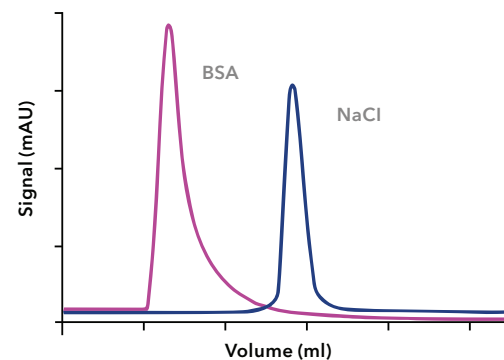
Group separation

Separation of small molecules from large molecules (e.g. **Desalting**)



High resolution separation

Separation of larger biomolecules within the fractionation range of the column matrix

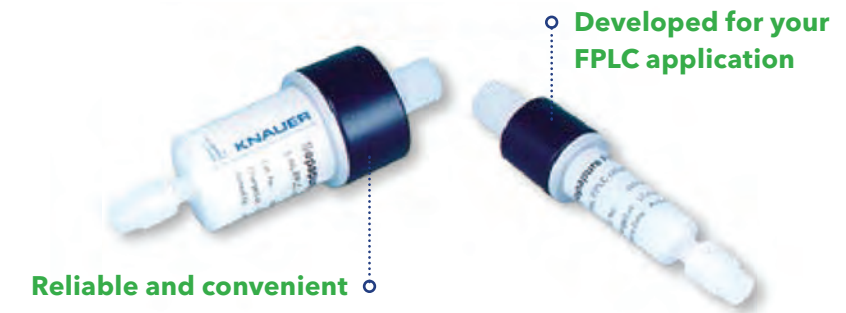


Sepapure® Desalting columns

Prepacked 1 ml or 5 ml columns

Key features

- Dextran based beads with particle sizes ranging from 20 - 50 µm
- 5 kDa exclusion limit (all molecules bigger than 5 kDa are not retained)
- Recommend flow rates: 0.5 - 2 ml/min (1 ml column); 1 - 5 ml/min (5 ml column)
- Maximum pressure: 3 bar
- Stored in 20% Ethanol



Affinity Chromatography (AC)

In affinity chromatography a highly specific interaction between the biomolecule of interest and the column matrix is resulting in the enrichment of the biomolecule at the stationary phase during the loading phase. Byproducts can be easily washed

off in the wash phase. The elution of the target biomolecule is realized by washing the column with a buffer including a high amount of competing ligand or low pH.

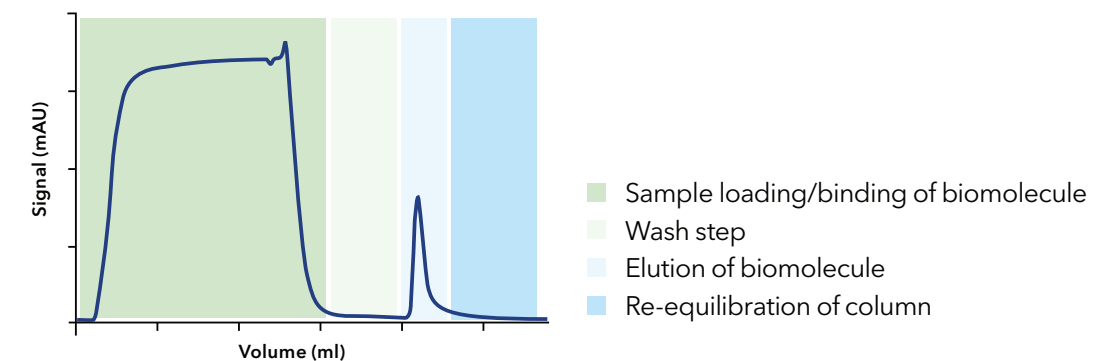
Recombinant tagged proteins

His - Tag via Ni-NTA column
GST - Tag via Glutathione column



Antibodies and antibody fragments

via Protein A immobilized on column
via Protein G immobilized on column



Sepapure® Affinity columns

Prepacked 1 ml or 5 ml columns

Key features

- Agarose based beads with particle size of 100 µm on average
- Static binding capacity: Ni-NTA < 40 mg/ml; Glutathione < 10 mg/ml; Protein A < 30 mg/ml human IgG; Protein G < 15 mg/ml human IgG
- Recommend flow rates: 0.5 - 2 ml/min (1 ml column); 1 - 5 ml/min (5 ml column)
- Maximum pressure: 3 bar
- Stored in 20% Ethanol

Ion-Exchange Chromatography (IEX)

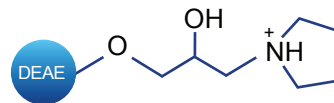
In ion-exchange chromatography biomolecules are separated according to their charge. Anion exchange is the method in which negatively charged molecules are binding to a positive matrix and in cation exchange positively charged biomolecules are binding to a negative column matrix. The bound molecules are released from the matrix by a gradual increase in ionic strength of the elution buffer.

Anion Exchange

Strong Anion Exchanger (Q)

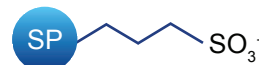


Weak Anion Exchanger (DEAE)

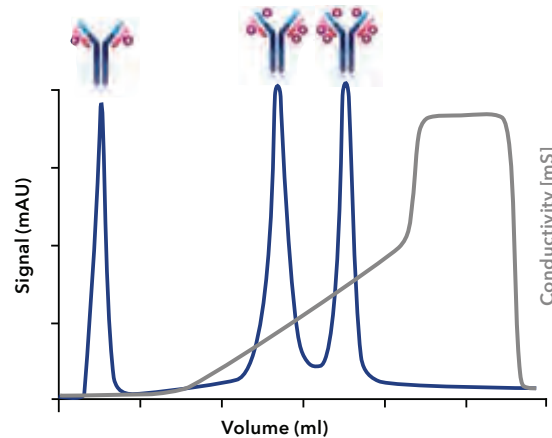
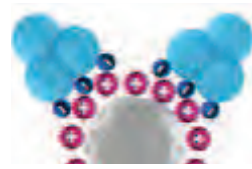
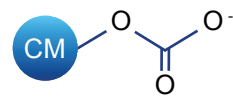


Cation Exchange

Strong Cation Exchanger (SP)



Weak Cation Exchanger (CM)

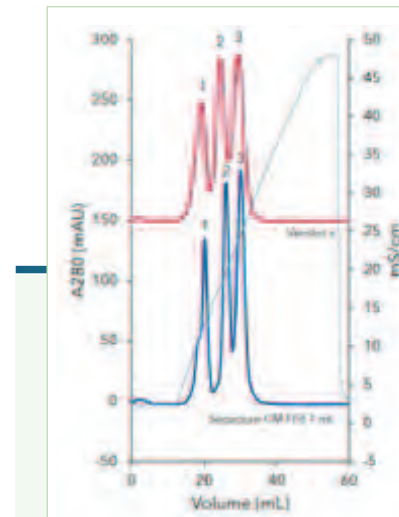


Sepapure® Ion-Exchange columns

Prepacked 1 ml or 5 ml columns

Key features

- Agarose based beads with particle size of 100 µm on average
- Ion capacity: < 0.12 mmol/ml
- Recommendend flow rates: 0.5 - 2 ml/min (1 ml column); 1 - 5 ml/min (5 ml column)
- Maximum pressure: 3 bar
- Delivered in 20% Ethanol



Comparison to other vendor

See information on detailed comparison of columns:
www.knauer.net/sepapure

Sepapure® bulk material

Next to prepacked FPLC columns KNAUER also offers FPLC bulk media for high performance purifications from lab to large-scale protein purification.

In addition to the media used with the prepacked FPLC cartridges we also offer SEC resins for high resolution separations.

Resin Type / Volume	5 ml	10 ml	25 ml	50 ml	100 ml	150 ml	250 ml	500 ml	1000 ml
Glutathione	•		•		•		•	•	•
Ni-NTA			•		•		•	•	•
Protein A	•		•		•		•	•	•
Protein G		•	•						
IEX-Resins			•		•			•	•
SEC 75			•	•	•	•	•	•	•
SEC 200			•	•	•	•	•	•	•

Sepapure® Size Exclusion media

Key features

- Cross-linked agarose-dextran composite with a particle size of 35 µm on average
- Maximum pressure: 3 bar (SEC 75) or 4 bar (SEC 200)
- Separation range of Sepapure SEC 75: 3 - 70 kDa
- Separation range of Sepapure SEC 200: 6 - 600 kDa
- pH tolerance: 2 - 14 (short term) / 3 - 12 (long term)

Detection

We provide a choice of UV/VIS detectors, ranging from single variable wavelength to 8-channel diode array detector with 3D scan capability.



Detector	UVD 2.1S	MWD 2.1L	DAD 2.1L
	Compact and cost-effective variable single wavelength UV/VIS detector	Reliable multichannel UV/VIS detector	Diode array detector for peak purity check
Wavelength	190-500 nm	190-700 nm	190-700 nm
Channels	1	4	8
3D scan	n/a	n/a	+
Integrable in ASM	+		

More UV detectors available for your applications: www.knauer.net/detectors



AZURA® Conductivity Monitor CM 2.1S

- Conductivity monitor for checking salt gradient
- Flow rates up to 100 ml/min
- 0.01 mS/cm-999 mS/cm
- pH option available

Caution!
May cause higher back pressure

Flow cells for CM 2.1S

Analytical	1/16"	10 ml/min	160 bar	30 µl volume
Preparative	1/16"	100 ml/min	100 bar	300 µl volume

AZURA® Detector RID 2.1L

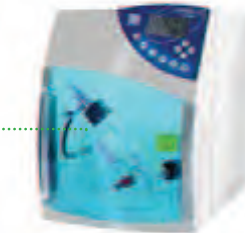
Refractive Index Detector for cost-effective, fast and reliable analysis of non-UV absorbent compounds.



Universal detection

A wide range of third-party detectors can be seamlessly integrated into AZURA® systems.

Gradient compatible and universal detection



Light scattering detector

Using the unique Low Temperature technology, this Evaporative Light Scattering detector LC allows universal high sensitivity detection of non-UV active substances.

Fluorescence detector RF-20A

The fluorescence detector RF-20A provides world-class sensitivity, excellent maintainability and diverse validation / support functions. It supports a wide range of applications from conventional to high-performance analysis.



Sensitive and selective fluorophor detection



The KNAUER interface box IFU 2.1 LAN allows highly precise analog data acquisition of third party modules over analog and relay outputs. Example: MALS-detectors for molecular weight determination.

Flow cells

Select from an impressive range of easily exchangeable flow cells which cover a wide range of application. Optional fiber optics technology offers the possibility to separate the flow cell spatially from the device providing enhanced security for hazardous, explosive or toxic work processes.

Fraction collection

Collect large quantities or large numbers of fractions

Manually - collection by direct control

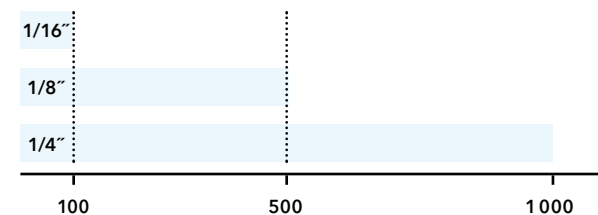
Volume-based - collection at defined volumes

Peak-based - collection according to detector signal

Fractionation valves

- Collecting large quantities
- From 6 to 16 fractions depending on the valve type
- Available as a single device or integrated into an Assistant ASM 2.1L for different flow rates

Fractionation valves max. flow rate (in ml/min)



Foxy Fraction collector

The Foxy R1 and Foxy R2 are versatile fraction collectors which fit to every purification need.

- Up to 125 ml/min for Foxy R1 and 1000 ml/min for Foxy R2
- Wide choice of racks from 96-well microplates up to bottles or funnels
- Double capacity for Foxy R2 with automatic rack recognition
- Active cooling for Foxy R1
- Supported in software Puritychrom®
- Stand-alone operation
- Repeated collection in same vials









Vario 4000 & Vario 4000 plus

The Vario 4000 is a more advanced fraction collector for demanding applications with high flow rates and a high number of fractions. Individual rack types are programmable. Just assemble your rack to your needs.

- For flow rates up to 1000 ml/min
- High number of fractions
- Supported in software Puritychrom®
- Standalone operation possible

Accessories

Accessory	Features	Benefit
 <p>Pressure Control</p>	<ul style="list-style-type: none"> • Contains two pressure sensors • Automatic determination of pressure difference with PurityChrom® • Connect 1/16" or 1/8" tubings • Up to 250 ml/min and 60 bar 	Monitor pressure over the column bed and protect column from damage
 <p>Air Sensor</p>	<ul style="list-style-type: none"> • Detect end of buffer or end of sample with PurityChrom® • Up to four air sensors per system • For transparent tubings with 1/16" or 1/8" or 1/4" outer diameter 	Protect column from air damage and support automation (e.g. sample injection)
 <p>AZURA® Click</p>	<ul style="list-style-type: none"> • Attach air sensor, pressure control, AZURA Organizer or your interface box to the side panel of your AZURA L device 	Organize your system.
 <p>AZURA® Organizer</p>	<ul style="list-style-type: none"> • Attach columns from 5 mm to 26 mm diameter, falcon tubes, a back pressure regulator or a pH flow cell 	Organize accessories directly at the system and reduce dead volume
 <p>Back pressure regulator (BPR)</p>	<ul style="list-style-type: none"> • Apply a constant back pressure to your system • Freely adjustable between 1-20 bar or 20-103 bar 	Prevent formation of air bubbles after the column which disturb detector signal
 <p>AZURA® Benchtop Rack</p>	<ul style="list-style-type: none"> • Install AZURA systems at space-limited sites, especially in cold rooms. 	Space-saving solution for AZURA system setup

AZURA® Bio purification systems

Product	Features	Page
AZURA Bio SEC 	0.001-10 ml/min, maximum 200 bar, injection valve sample for sample loops, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software	22
AZURA Bio AC 	0.01-50 ml/min, maximum 200 bar, selection valve for 6 buffers/samples, variable single wavelength UV-detector, fraction valve for 5 fractions and waste, PurityChrom® software	23
AZURA Bio Lab 	0.001-50 ml/min, maximum 200 bar, injection valve sample for sample loops, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software in basic configuration. Configure your FPLC system based on your purification requirement.	4
AZURA Bio Lab Two-step purification 	0.01-50 ml/min, maximum 200 bar, sample injection via sample loop or sample pump, automated storage and reinjection of proteins, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software	24
AZURA Bio Pilot 	Up to 1000 ml/min, sample pump for large sample volumes, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software in basic configuration. Configure your FPLC system based on your purification requirements. Scale-up is possible with same flexibility, software but minimal footprint.	5

Components from lab to pilot

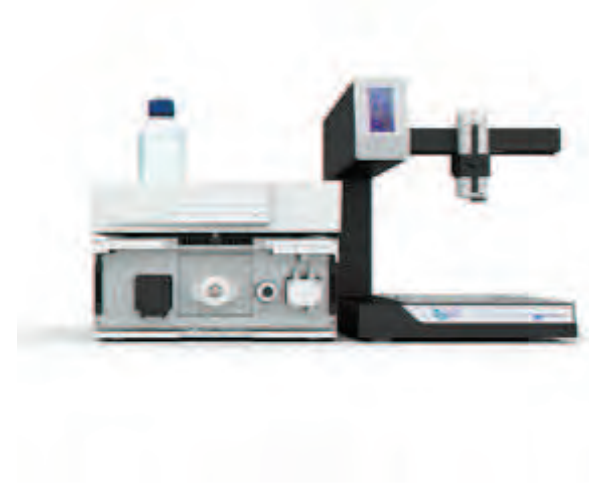
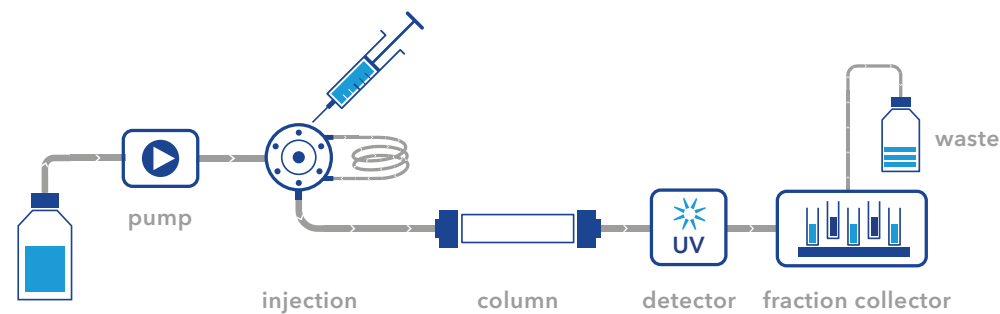
Product	Features	Page
Buffer delivery		
Compact pump	10 or 50 ml/min, isocratic	6
Gradient pump	10 or 50 ml/min, quaternary: selection of 4 buffers (A, B, C, D) Binary: selection of 2 buffers (A1, A2, B1, B2)	7
Scale-up pump	100, 250, 500, 1000 ml/min, binary to quaternary gradient	7
Extended buffer selection	With additional valves each for 8 buffers	6
Sample selection	For maximum 8 samples	10
Columns		
Column selection valves	For 2 columns, 5 columns and 5 columns with reverse flow option	11
Sepapure® columns and media	Columns and media for Affinity, Size Exclusion and Ion-Exchange Chromatography	12
Detection		
Wide choice of detectors	Variable single wavelength UV, multiple wavelength UV, full spectra diode array (DAD/3D Spectrum), conductivity and pH monitor, fluorescence, refractive Index	16
Fraction collection		
Fractionation valve	For 6 to 16 fractions, depending on the valve type with flowrates up to 1000 ml/min	18
Fraction collector	From 96-well microplates up to bottles or funnels, up to 1000 ml/min	18
Sample injection		
Injection valve	1/16" tubing: up to 50 ml/min 1/8" tubing: up to 500 ml/min	10
Sample pump	10 or 50 ml/min	10
Autosampler	Up to 10 ml injection volume, from microtiter plates to 10 ml vials	10
Software		
PurityChrom® software	Highly flexible method writing, intuitive user-interface, volume- or time-based, with special features like system visualisation, hold & adjust option, extended threshold functions, check for impurities	26
Safety features		
Accessories for protection and automation	Air sensor, pressure control, back-pressure regulator, leak management, mounting solutions	19

AZURA® Bio SEC system

Time consuming gel filtration runs?

AZURA Compact SEC systems take over time-consuming SEC methods in your lab without blocking your valuable FPLC system. Thanks to its compact design and intuitive FPLC software PurityChrom®, the system offers outstanding performance and

ease of use. Pre-designed methods are included in the software and can be easily adapted by changing the column volume. AZURA Compact SEC supports all columns available on the market.



Key features

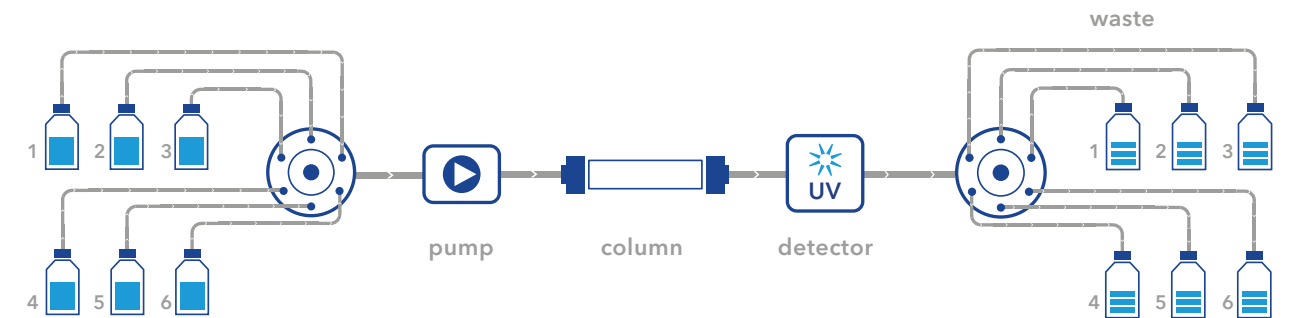
- Flow rate: 0.001-10 ml/min; 0.1-8.0 ml/min (recommended)
- Maximum system pressure: 150 bar
- Injection valve for sample injection via sample loop
- Variable single wavelength UV-detector (190-500 nm)
- Fraction collector for fractionation
- Columns from all vendors can be used
- PurityChrom® software

AZURA® Bio AC system

For affinity chromatography

The AZURA Compact AC system qualifies for fast and reliable affinity chromatography. Select your sample, your washing and elution buffer using

the selection valve. Your proteins of interest are detected by UV and automatically collected via the fractionation valve.

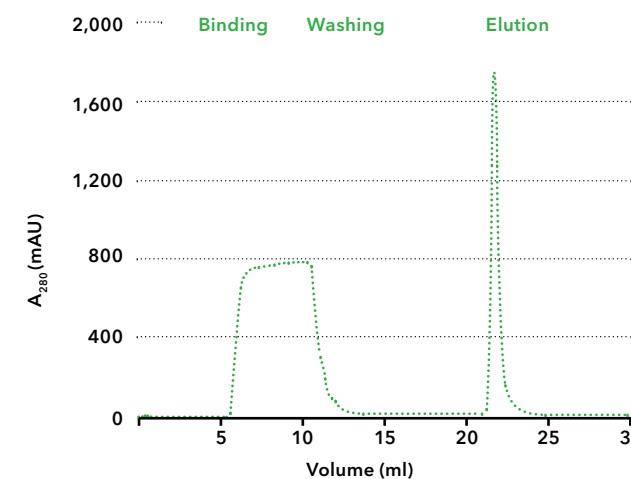


Use the selection valve for your buffers and sample.



Key features

- Automatic sample/ buffer selection valve for up to 6 buffers or samples
- Fraction valve (6 ports) for fractionation
- Flow rate: 0.01 - 50 ml/min; 1 - 40 ml/min (recommended)
- Variable single wavelength UV-detector (190 - 500 nm)
- Columns from all vendors can be used
- PurityChrom® software
- Maximum system pressure: 150 bar



Protein purification based on high affinity Chromatogram & Legend

Special configuration Two step purification

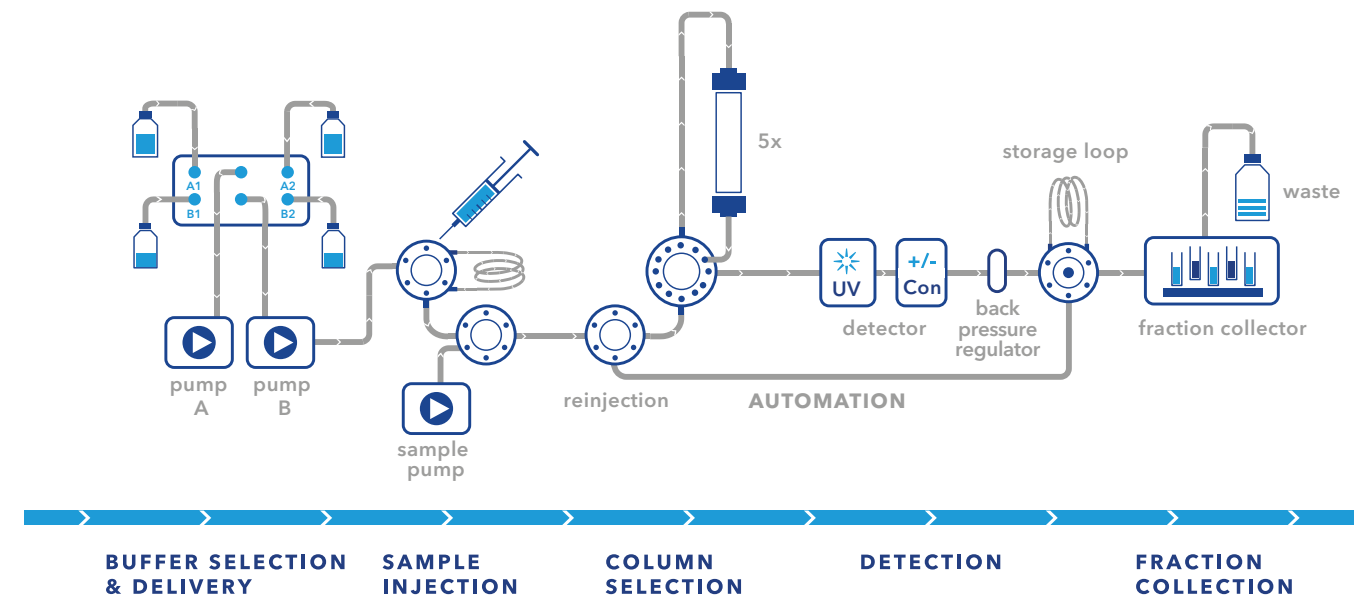
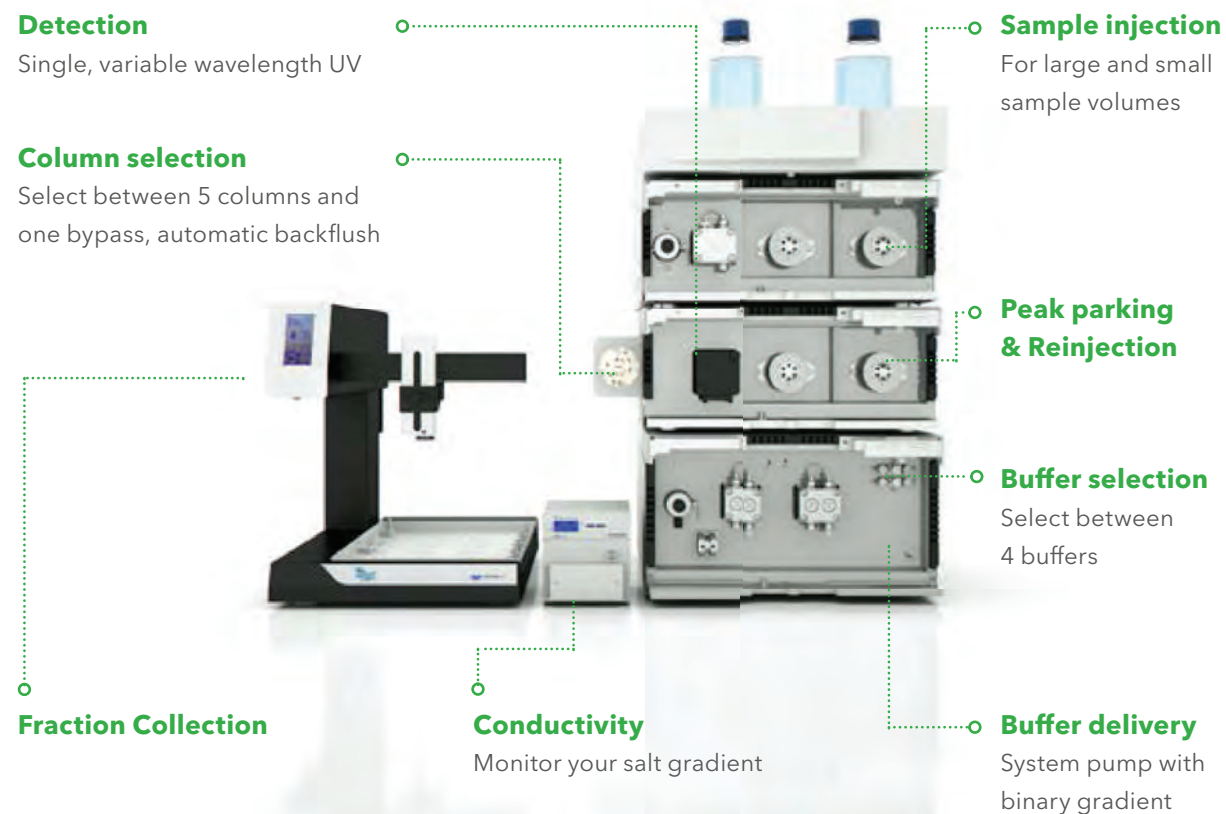
Special multicolumn chromatography solutions

Protein purification involves most of the times two to three steps:

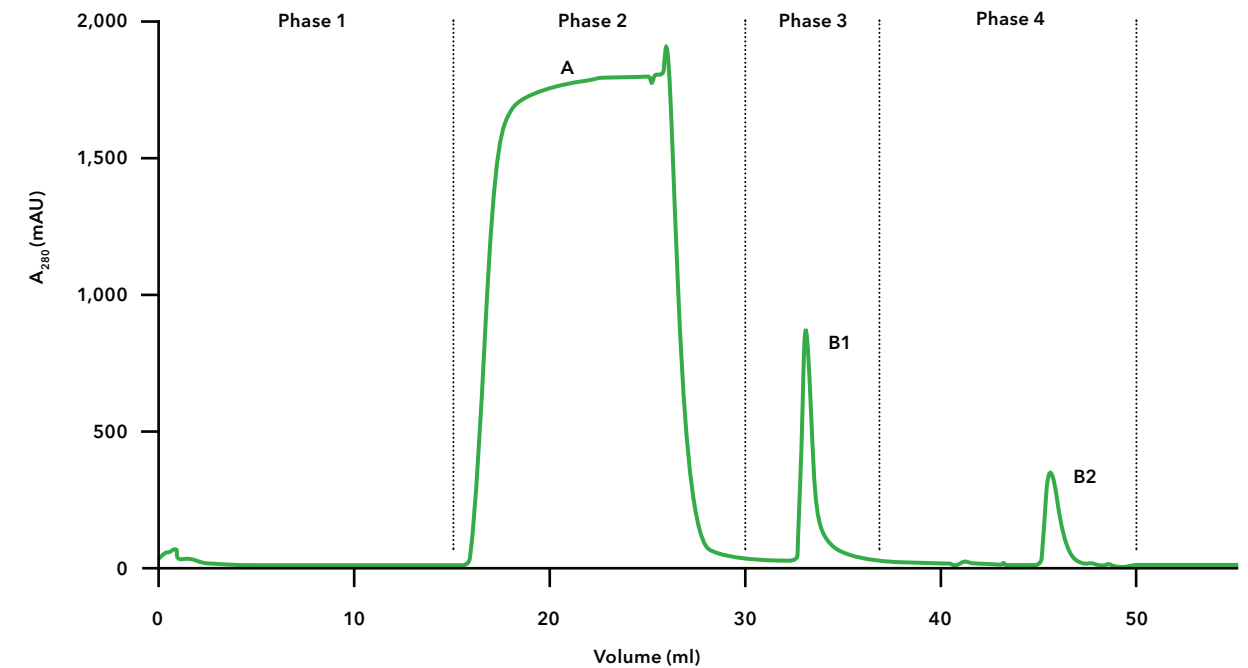
1. capture step
2. optional intermediated step
3. polishing step

The transition from one to another step generally involves manual interaction and thus is time consuming. Automation by combining these steps increases the efficiency and optimizes the workflow. The quick and automated linkage of multiple

chromatographic purification steps into one method eliminates manual sample handling and minimizes time spent between steps. This automation strategy can be easily adapted to each purification task.



Automated two-step purification of mouse IgG antibodies

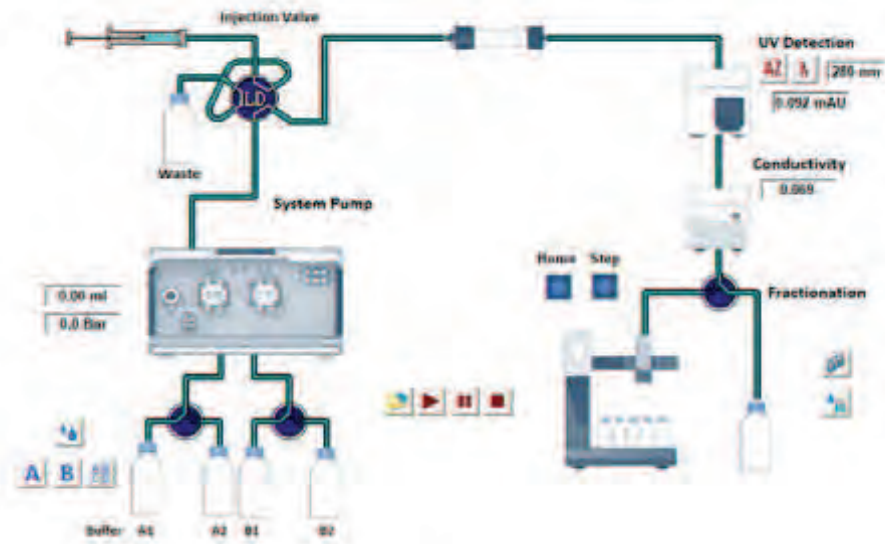
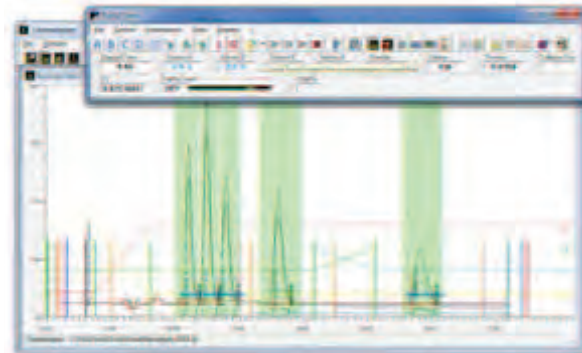


The affinity chromatography step was automatically combined with a gel filtration step to exchange the buffer of the purified mouse IgG antibodies; Phase 1: Column equilibration, Phase 2: Sample injection and washing, Phase 3: Elution of IgG from protein A column, Phase 4: Desalting of IgG

Control your purification

PurityChrom®

PurityChrom is a powerful software to control your FPLC system. Get familiar with PurityChrom in shortest time and with no effort due to the intuitive and clearly structured user interface. Choose a time- or volume based workflow by just clicking one button. Create methods with highest flexibility to realize complex application without losing easy handling. Offline licenses for creating methods and data evaluation are for free.



System visualization

Keep an eye on your system with the system visualisation. The interactive flow path allows to control your system. Switch valves, start pumps, set autozero, start fraction collection.

Hold & adjust (a running method)

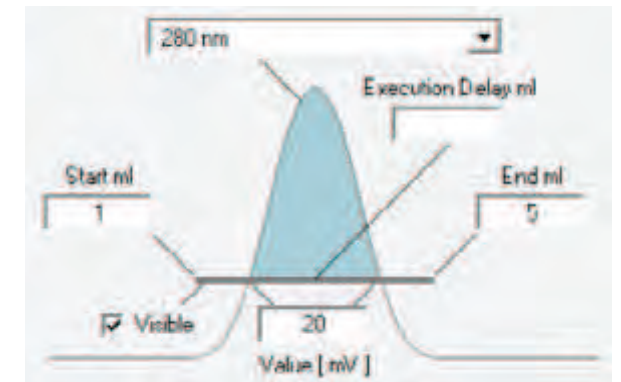
You have full control of your run. Hold a run to adjust the method or the system. Stay always in control and change the parameters of a running method.



Extended threshold functions

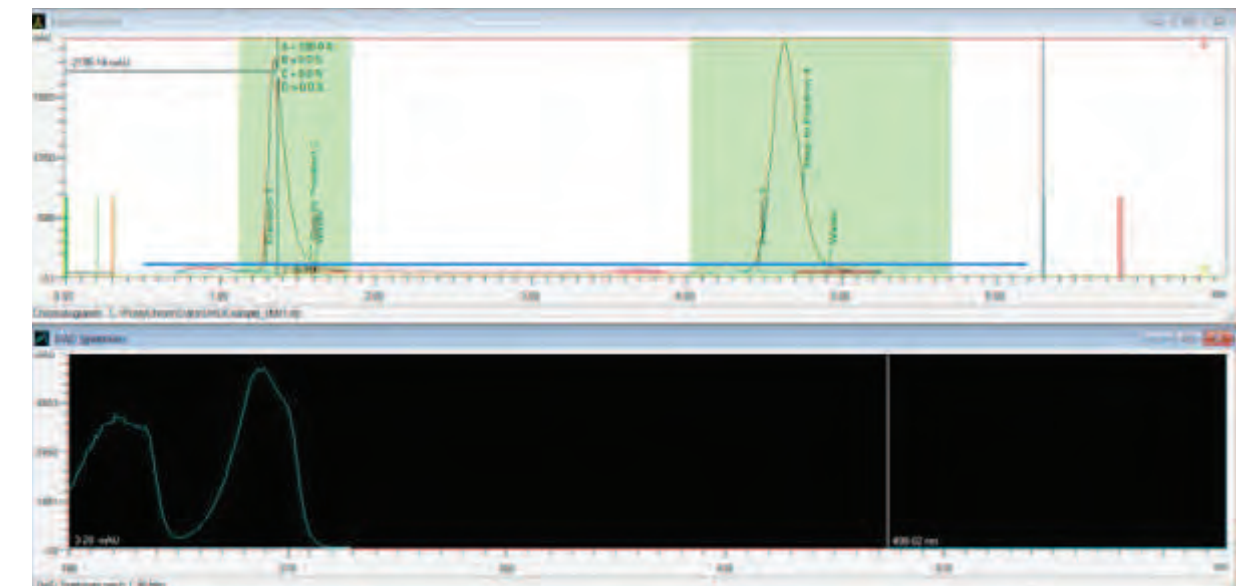
Automate any software function triggered by signals of any channel.

Automatically start fraction collection at the beginning of your desired peak. Protect the system from overpressure and air bubbles. After end of sample detection the software offers the possibility to automatically start or continue the run. Automate the whole purification starting from sample injection, via column washing to elution.



Check for impurities - full spectra diode array (DAD)

Check the purity of your peaks based on the absorbance spectra anywhere in the elution profile.



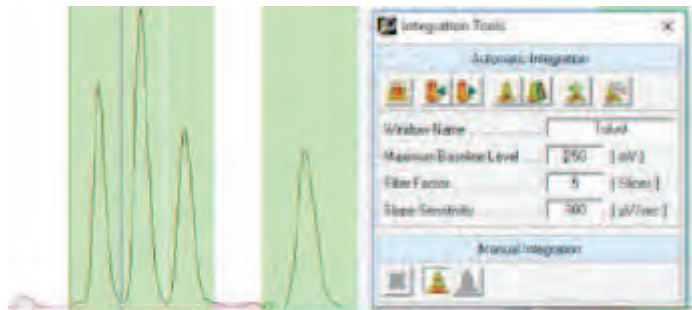
Tutorials on YouTube

Get familiarized with manually controlling your system, writing methods and analyzing your data using PurityChrom®.
www.youtube.de/KNAUERhplc



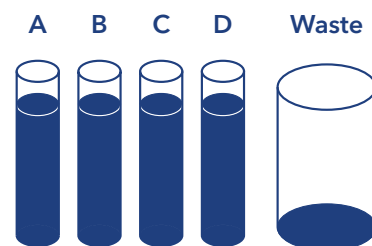
Intuitive data analysis

Integrate peaks fully automatically or manually. Receive the peak results by clicking on one button.



Solvent supply - calculate the consumption of buffers

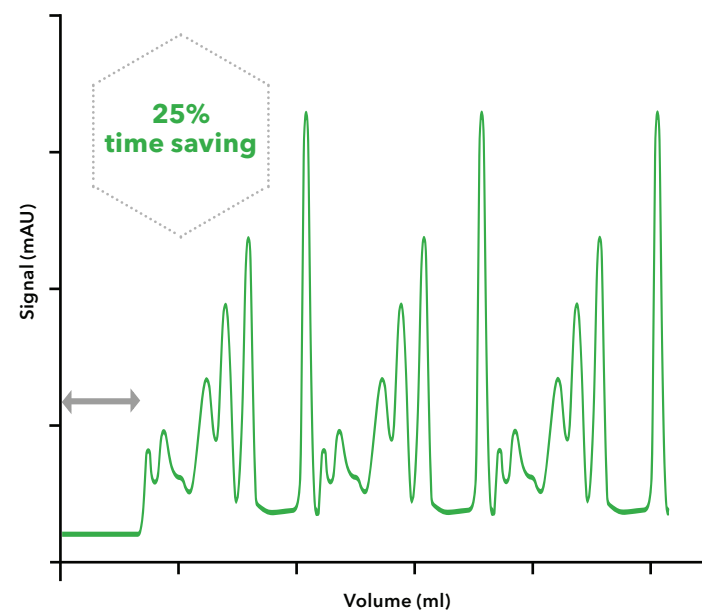
The solvent supply function calculates the consumption of buffers and the waste level for the current run, thus preventing the column from running dry and flooding the lab.



Stacked Injection

Size exclusion chromatography separates the proteins according to their size. After selection of SEC medium, sample volume and column dimensions are the two most critical parameters that will affect the resolution of the separation. For most SEC runs the sample volume should not exceed 2% of the total column volume to achieve maximum resolution.

For larger sample volumes the sample must therefore be divided into different runs. However, this takes a lot of time and is not very efficient. With the stacked injection function in PurityChrom it is possible to run different runs automatically one after the other. The injection of the next run takes place during the current run, so that the time until the elution of the first peak can be fully exploited. This increases efficiency and saves time.



Customer review

AZURA® Bio purification solution by KNAUER

„Our KNAUER FPLCs are the workhorses in the lab.“

“My lab studies the structure and function of membrane proteins. Due to the inherent instability of these proteins we purify them in the cold room. We needed robust FPLCs with good pumps that tolerated these conditions well.

In addition, the systems needed to be easy to maintain. Knauer provided us with skilled advice on virtually every component of the system, ranging from tubing and pumps up to the software. Consequently, our systems are perfectly tailored to our needs. Most of the maintenance we can do ourselves. For remaining questions, we can rely on the great support Knauer offers. Our Knauer FPLCs are the nononsense workhorses in the lab. I highly recommend Knauer.”



Jun. Prof. Dr. Eric R. Geertsma
Institute of Biochemistry,
Goethe-University Frankfurt

Photo: Uwe Dettmar

System components

- AZURA® UV Detector UVD 2.1S
- AZURA® Valve Drive V 2.1S
- AZURA® Pump P 4.1S
- Foxy fraction collector



AZURA Compact SEC systems take over time-consuming SEC methods in your lab without blocking your valuable FPLC system.

Contact us:

sales@knauer.net

Science Together



Based in Berlin, KNAUER is a medium-sized, owner-managed company that has been serving the sciences since 1962. We develop and manufacture scientific instruments of superior quality for liquid chromatography. The range includes sys-

Independent and family owned



The founder Dr. Herbert Knauer and his wife Roswitha are still active as advisers in the company to this day. The couple's daughter, Alexandra Knauer, has been managing director and



owner of the company since 2000. Several awards for outstanding products and innovations as well as entrepreneurial excellence make KNAUER a „leading employer“.

**We separate molecules
and unite people.**

www.knauer.net



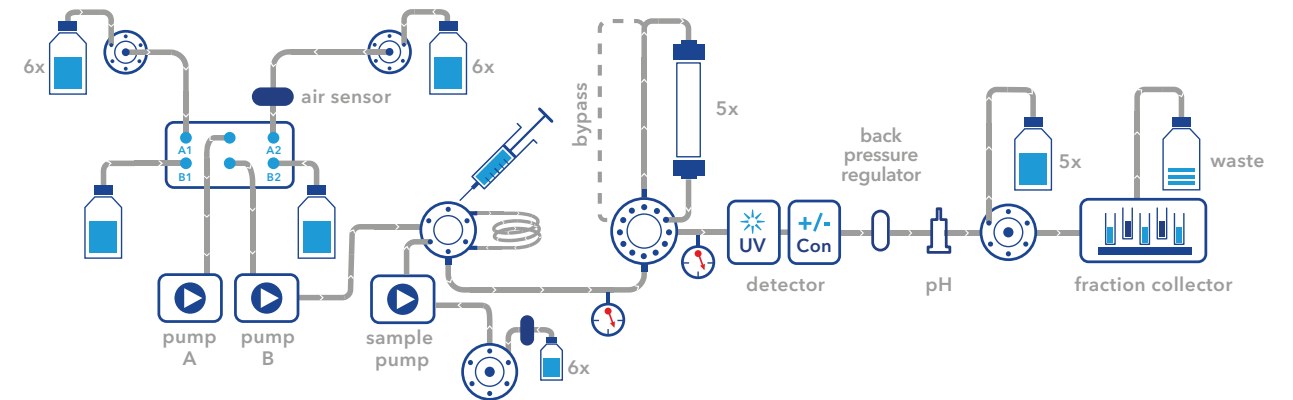
Worldwide partner in science since 1962

tems and components for analytical HPLC / UHPLC, preparative HPLC, fast protein liquid chromatography (FPLC), multi-column chromatography / simulated moving bed (SMB), and osmometry.

System configurator Bio purification by KNAUER

METHOD

- SEC**
Size Exclusion Chromatography
- AC**
Affinity Chromatography
- IEX**
Ion-Exchange Chromatography
- HIC**
Hydrophobic Interaction Chromatography



BUFFER SELECTION & DELIVERY

- 10 ml/min binary gradient pump P 6.1L
- 10 ml/min quaternary pump P 6.1L
- 50 ml/min binary gradient pump P 6.1L
- x 100 ml/min pump P 2.1L
- x 250 ml/min pump P 2.1L
- x 500 ml/min pump P 2.1L
- x 1000 ml/min pump P 2.1L
- Ternary gradient module for pump P 2.1L
- Binary gradient module for pump P 2.1L

- x Buffer selection valve (6 further inlets)
- x Buffer selection valve (8 further inlets)

SAMPLE INJECTION

- x Injection valve
- Sample pump module
- Sample selection valve: x inlets
- Biocompatible Autosampler AS 6.1L

COLUMN SELECTION & THERMOSTAT

- Column selection valve up to 50 ml/min (5 columns, one bypass, reverse flow)
- Column selection (two columns or one bypass)
- Column selection high flow (5 columns, one bypass)
- Column selection high flow (5 columns, one bypass, reverse flow)

COLUMNS & MEDIA

- SEC:** Desalting ml
- SEC:** SEC 75 ml
- SEC:** SEC 200 ml
- AC:** Protein A ml
- AC:** Protein G ml
- AC:** Ni-NTA ml
- AC:** Glutathione ml
- IEX:** DEAE - Weak anion exchange ml
- IEX:** CM - Weak cation exchange ml
- IEX:** Q - Strong anion exchange ml
- IEX:** SP - Strong cation exchange ml

DETECTION

- UV/VIS single wavelength
- UV/VIS multiwavelength
- Conductivity
- pH
- Fluorescence
- Refractive index
- Light Scattering
- Analog integration of further detectors

FRACTION COLLECTION

- Fractionation valve
- Foxy fraction collector with fixed rack types
- Labocol fraction collector with individual rack types
- Rack for fraction collector

ACCESSORIES

- x Air sensor main pump
- x Tubing 1/16"
- x Air sensor feed pump
- x Tubing 1/8"
- Pressure control (2 pressure sensors)
- x Tubing 1/4"
- x Back pressure regulator
- Workstation (Windows)
- AZURA Organizer

Science Together



AZURA[®] SMB systems

Chromatography for continuous separations



Why better choose SMB instead of batch chromatography

SMB chromatography is a HPLC technique for the separation of binary mixtures with high productivity and purity.



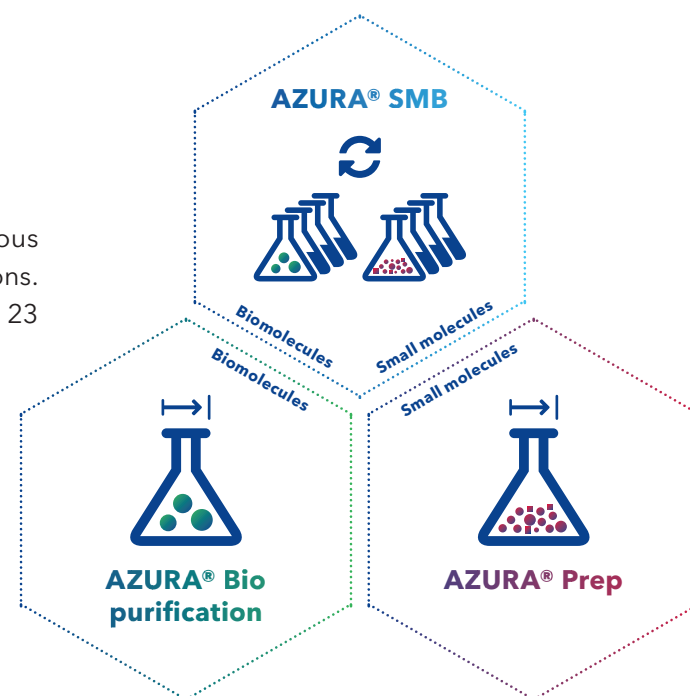
Get higher productivity and purity than with comparable batch systems – even with a smaller system.

Save up to 90% of the solvent and reduce the solid phase costs up to 80%

Gain nearly undiluted product and minimize concentration efforts.

AZURA® purification systems

KNAUER offers system solutions for continuous separation tasks as well as for batch separations. Visit us online or take a look at page 22 and 23 for more information.



Introducing SMB chromatography

Simulated moving bed chromatography (SMBC) is increasingly applied as a separation technique in the pharmaceutical industry, production of fine chemicals and in the field of bioengineering. SMB is a method in process chromatography that enables substance mixtures to be continuously separated and extracted in two fractions.

By repeated use of the SMB process each partial fraction can be separated into a further fraction – down to binary substance mixtures. Typically, the SMB process is set up in advance for a two component mixture. Following this, both substances can be immediately extracted in pure form.

What is the difference between batch LC and SMBC?

Batch chromatography (single-column)	SMB chromatography (multi-column)
Unlimited number of fractions	Two fractions, no waste
Recovery typically below 80%	Recovery up to 100%
EITHER high purity OR high yield	High purity AND high yield
Isocratic or gradient	Isocratic
High solvent consumption	Can be as low as 10% of batch consumption
Very diluted product	Product concentration comparable with input concentration (feed)

Limited to binary mixtures?

The SMB process is ideally suited for two-component separations (Fig. 1a). For the task of separating and collecting multiple fractions, classical batch LC might be the better option (Fig. 1b).

SMB chromatography can also be used for the separation of more than two peaks (multi-component mixtures). Therefore it is possible to “split the chromatogram” at a certain point (Fig. 1c).

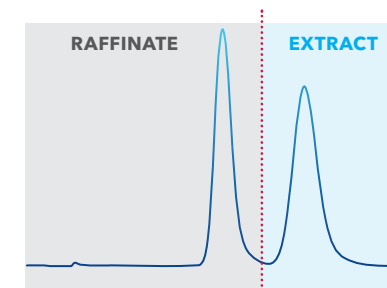


Fig. 1a: Basic binary mixture for a SMB separation.

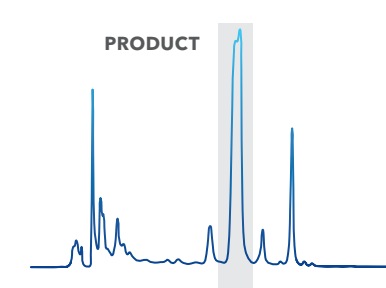


Fig. 1b: Typical multi-component mixture for classical batch chromatography.

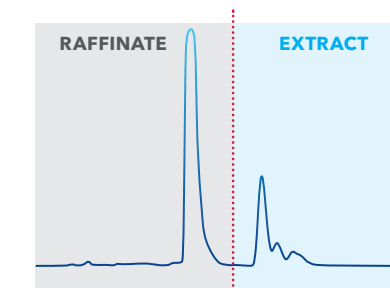


Fig. 1c: Multi-component mixture, can be separated in two different fractions with SMB.

PurityChrom® MCC

Intuitive and highly functional control software

Flexibility

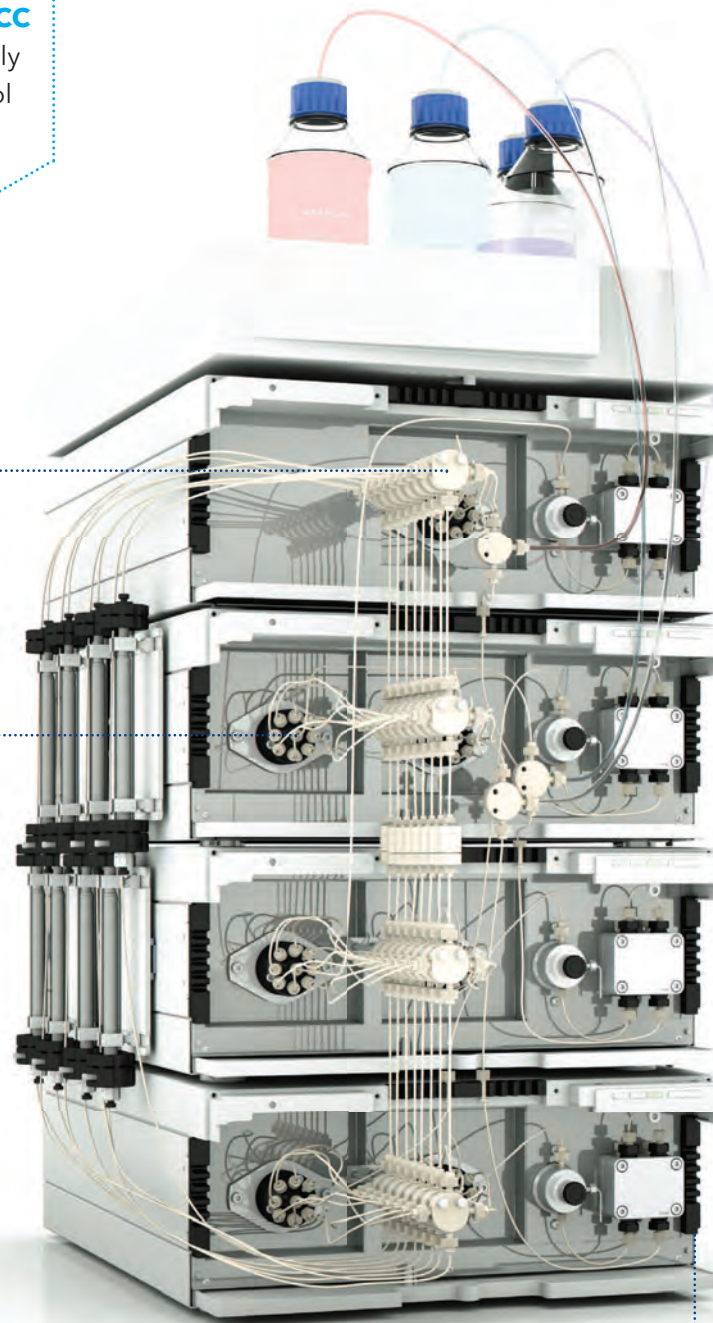
Up to 8 columns at max. 130 bar

Multi-position valve

Standard valves for flexible zone definition and low maintenance costs

Temperature control

Columns can be heated or cooled (requires additional equipment).



Gram scale

Flow rates up to 50 ml/min and columns up to 30 mm ID allow to increase your throughput up to several hundred grams.

Small footprint

The AZURA® SMB systems require little space on the lab bench.

Biocompatible

Fully biocompatible version available. Perfect solution for the continuous purification of biomolecules like proteins.

AZURA® SMB Lab

This SMB system is optimized for separation tasks on a scale of several hundred grams. The standard configuration consists of four AZURA® assistants ASM 2.1L with seven multi-position valves and four AZURA® pumps P 4.1S as well as our user-friendly software PurityChrom® MCC including required IT hardware. Depending on the special

requirements of every separation the SMB system can be freely configured via valve switch (e.g. closed-loop, open-loop, 3-zone) and is upgradable with detectors and flow meter. See table for available configurations of the AZURA® SMB Lab. Individual configuration is available on request.

Available configurations for AZURA® SMB Lab

Max. flow rate	Number of columns	Max. pressure	Art. No.	Description
10 ml/min	6	300 bar	A29002	Ultra high pressure SMB stainless steel (sst)
50 ml/min	8	130 bar	A29001*	Stainless steel
	8	130 bar	A29000*	Biocompatible (PAEK, ceramic)

* Standard configuration

PurityChrom® MCC
Intuitive and highly functional control software

Wide field of applications
Up to 8 columns at max. 100 bar

Kilogram scale
Flow rates up to 500 ml/min allow very high throughput on a kilogram scale



Temperature control
Columns can be heated or cooled (requires additional equipment)

Biocompatible
Fully biocompatible version made of titanium and PEEK on request

Multi-position valve
Standard valves for flexible zone definition and low maintenance requirements

AZURA® SMB Pilot

The AZURA® SMB Pilot is designed for the separation of binary mixtures on a hundred gram to kilogram scale and is typically used with columns up to 50 or 100 mm ID. Its special emphasis is put on the continuous operation mode and highest productivity.

The SMB standard configuration consists of four AZURA® pumps P 2.1L and seven 8-port multi-position valves integrated into four AZURA® assistants ASM 2.1L. Our user-friendly software PurityChrom® MCC and the required IT hardware are also included. We offer several variations of the standard system configuration.

Available configurations for AZURA® SMB Pilot

Max. flow rate	Number of columns	Max. pressure	Art. No.	Description
100 ml/min	8	100 bar	A29504	Low dead volume (1/16", stainless steel)
	8	100 bar	A29502	Stainless steel
500 ml/min	8	100 bar	A29501*	Stainless steel

* Standard configuration

Upgrade kits for AZURA® SMB systems

Heating and column organisation

Save space and time with our SMB oven or multi-column stands



Easy access to columns, less bench space required



A29902

Up to 120°C



Full control over the column temperature

Up to eight KNAUER columns (max. 50 mm ID, 250 mm length)



A29901

A29900

Description	Art. No.
Oven for AZURA® SMB Lab and AZURA® SMB Pilot systems (8 KNAUER columns with up to 250 × 50 mm inner dimension)	A29900
Column holder for 8 SMB columns with up to 250 × 50 mm	A29901
Column holder for 8 SMB columns with 8, 16 mm ID and up to 250 mm length or 20 mm ID and 150 mm length	A29902

Batch-upgrade

Add a batch system into your SMB

Upgrade your system and perform simple separation tasks or estimate your separation parameters directly without buying an additional batch LC system. The Batch-Upgrade kits include a detector and an injection valve.

Flow rate	Material	Art. No.
up to 100 ml/min	Stainless steel	A29601
	Biocompatible	A29600
up to 500 ml/min	Stainless steel	A29603
	Biocompatible	A29602

Installation

Standard installation and familiarization - including system installation, instruction to system and software as well as general considerations for SMB operation.

	Art. No.
Europe	A0000SMBEU
International	A0000SMBIN

System and process control

High-accuracy mass flow meters for highest process stability

Number of mini-CORI-FLOW™	Position	Monitors	Monitoring level	Field of application
1	Column	Process stability	+	Process evaluation; standard systems
2	Column; feed pump	Process stability; feed flow	++	Process evaluation; demanding separations; expensive feed; low feed flow
4	At every pump	Pump flow	++++	Production process; complete control over system stability

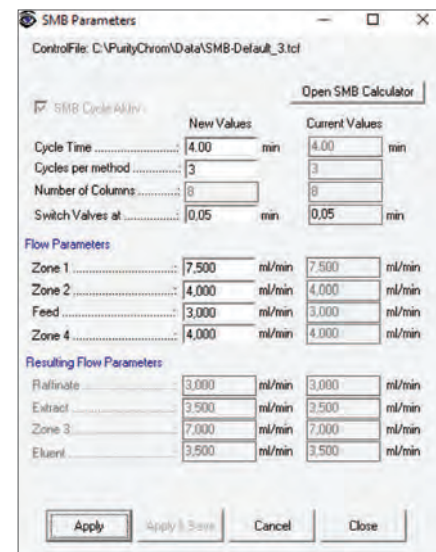
Description	Art. No.
One mini CORI-FLOW™ M13 for AZURA® SMB Lab, incl. accessories	A29800
Four mini CORI-FLOW™ M13 for AZURA® SMB Lab, incl. accessories	A29801
One mini CORI-FLOW™ M14 for AZURA® SMB Pilot, incl. accessories	A29802
Four mini CORI-FLOW™ M14 for AZURA® SMB Pilot, incl. accessories	A29803

PurityChrom® MCC

Software for multi-column chromatography (MCC)

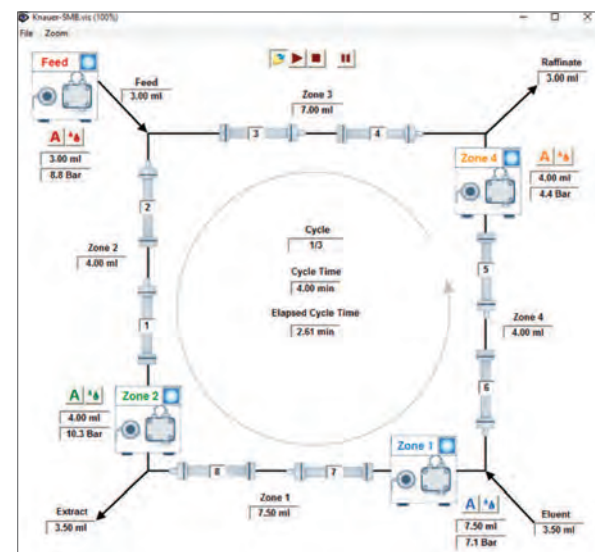
SMB Parameter Wizard

Insert your calculated adsorption parameters. The SMB Parameter Wizard will calculate the operation point. The parameters can easily be transferred to the SMB method file.



System visualization

The system visualization is everything you need to control the SMB system. The visualization can be freely configured. Keep track of all the information.



Software for multi-column chromatography (MCC)

System monitor

Monitor as many channels as necessary at once and keep full control of your SMB process all the time.



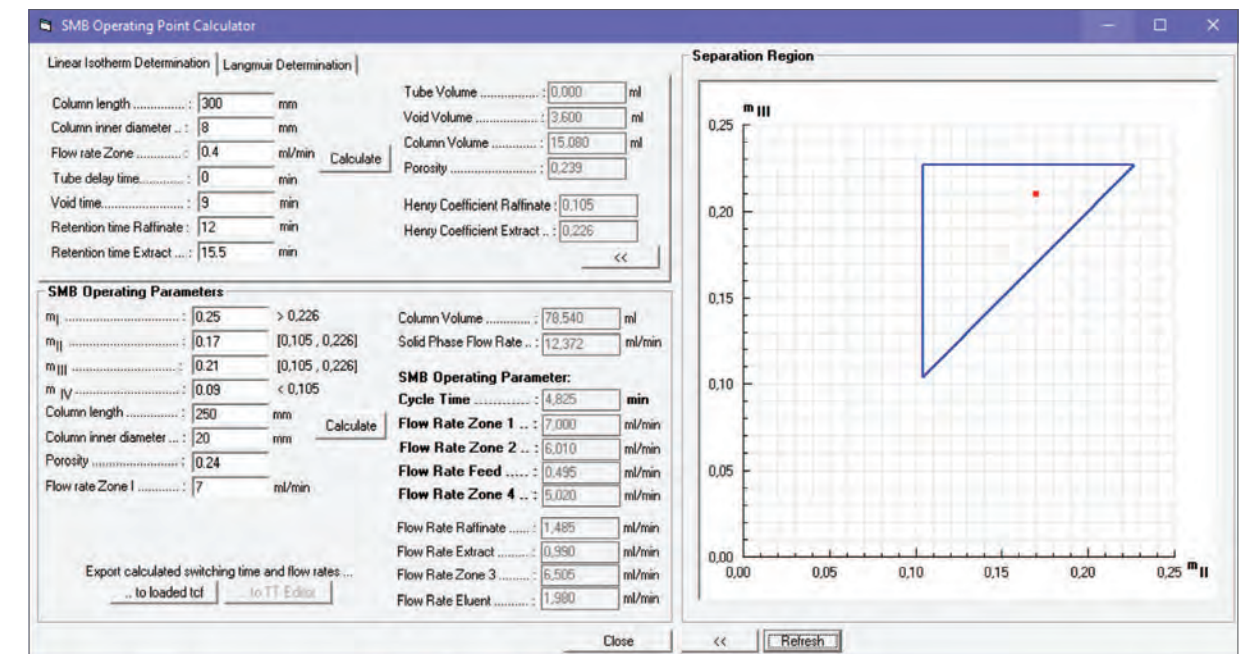
Security options

The user management ensures the integrity of your separation methods. In addition every change made through the method is protocolled by the software and saved in the result files. Our PurityChrom® software is 21 CFR part 11 compliant.

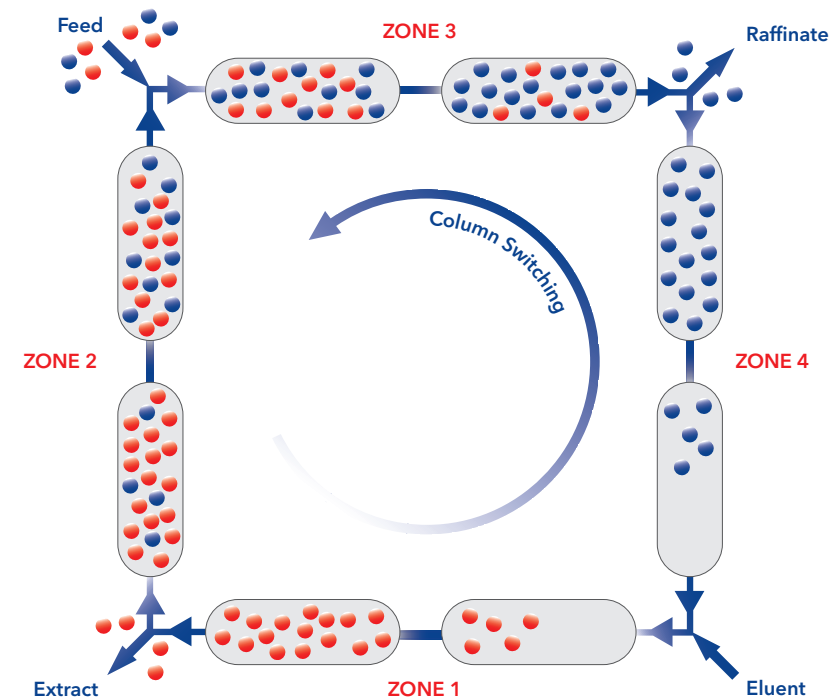
Time [min]	Function	Parameter
0.00	Feed	100.0, 0.0, 0.0, 0.0 3.00 ml/min Constant Flow
0.00	Zone 4	100.0, 0.0, 0.0, 0.0 4.00 ml/min Constant Flow
0.00	Pressure Pumps	Maximum Pressure Pump 1 = 150.0 Bar
0.05	Valve Position	All Valves = Next Position
0.10	Start Chromatogram	Channel 1,2,3,P1,F1,P2,F2,P3,F3,P4,F4 (500 ms)
0.55	Valve Position	All Valves = Next Position
1.05	Valve Position	All Valves = Next Position
1.33	Zone 2	100.0, 0.0, 0.0, 0.0 4.00 ml/min Constant Flow
1.55	Valve Position	All Valves = Next Position
2.05	Valve Position	All Valves = Next Position
2.55	Valve Position	All Valves = Next Position
3.05	Valve Position	All Valves = Next Position
3.55	Valve Position	All Valves = Next Position
4.40	Stop Chromatogram	All started Channels
4.50	Stop all	

SMB Operation Point Calculator

Adsorption isotherms can be entered into an integrated starting point calculator. The generated values can be checked via a visual feedback very easily. The parameters will be transferred directly into the SMB Parameter Wizard.



The SMB principle



SMB process scheme

The SMB process enables the separation of binary mixtures by means of a simulated countercurrent between the solid and liquid phases. This is accomplished with a series of chromatography columns arranged in a ring. An eluent flow circulates through this ring. Two inlets (for feed and eluent) and two outlets (extract/red and raffinate/blue) define four separation zones. By continuously feeding sample and synchronously switching the columns against the eluent flow direction, a countercurrent is achieved between the solid and liquid phases, leading to high purity of both target fractions. The movement of the solid phase is realized by simultaneously switching seven multi-position valves (AZURA® SMB) or one central multi-position valve (former KNAUER SMB, CSEP®).

The bulk of the eluent is continuously circulated in the system, making it necessary to replace only that small amount which is removed in the extract and raffinate, thereby enabling savings of up to 90% of the eluent in comparison to a batch process. Due to the simulated countercurrent, the stationary phase is significantly better utilized with the SMB technique as compared to the batch process technique. The number of theoretical plates might be also less important, making it possible to use cost-effective larger particle size for the stationary phases.



AZURA® SMB Lab manifold, stainless steel version.

CSEP® versus AZURA® SMB

What are the differences between the previous KNAUER CSEP® system and the new AZURA® SMB?

	CSEP® (Former KNAUER SMB) (C9116/C9812)	AZURA® SMB (Lab/Pilot)
Max. flow rate [ml/min]	50/500	50/500
Max. pressure rating [bar]	100/50	400/344
Max. number of columns	16/12	8/8
Max. temperature	60°C	60°C
Dead volume	Very low	Low
Flexibility	Medium	Very high
Choice of wetted material (valve)	None	Variable, different options for every application (e.g. stainless steel, ceramic)
System visualization	No	Yes

How AZURA® SMB works

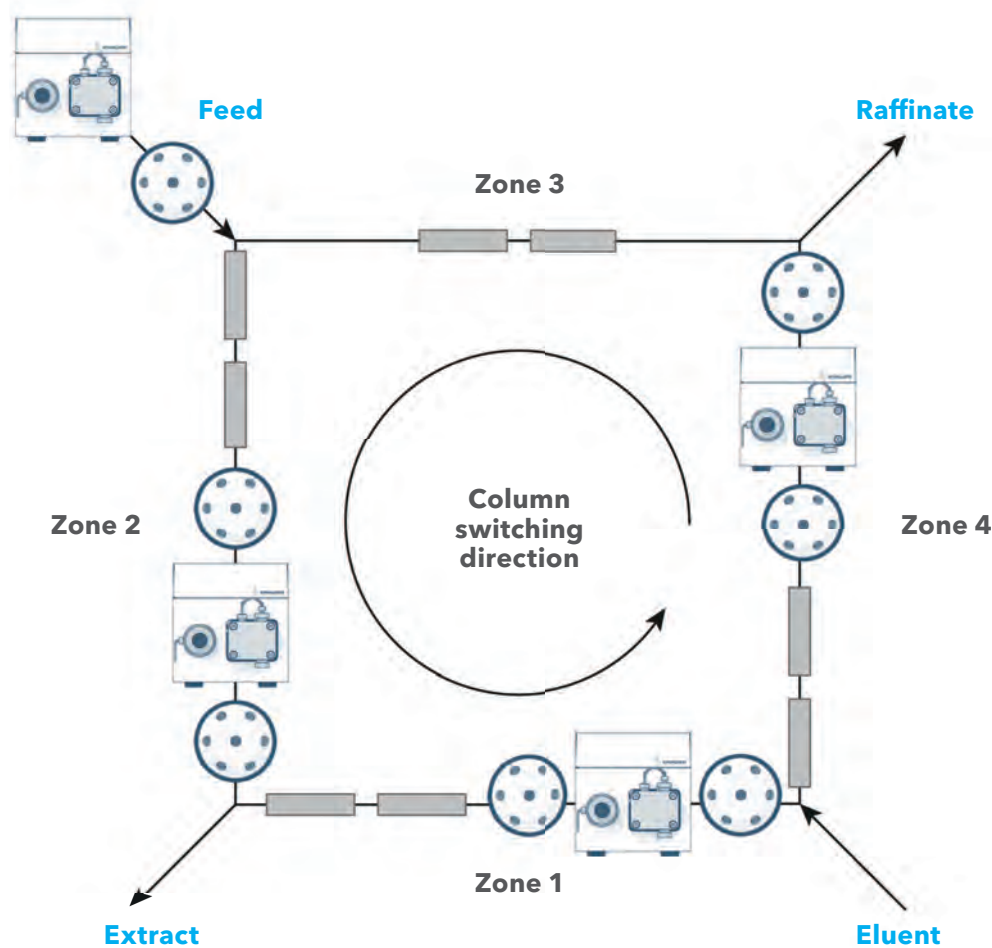
System configuration

The standard AZURA® SMB systems consists of four pumps and seven multi-position valves.

The devices are arranged as follows:

- Three pumps (Extract, Raffinate, Eluent) are placed inside the SMB cycle.
- The feed pump is placed outside the SMB cycle.
- Four valves are placed at the pump outlets.
- Three valves are placed at the pump inlets. The feed pump inlet is not connected to a multi-position valve.

Due to this configuration the SMB system can be used very flexible for many different separation modes. Additionally to the process stability AZURA® SMB systems are outperforming every other SMB system on the market regarding material and configuration flexibility as well usable pressure range.



Schematic AZURA® SMB design

Where can a SMB separation be used?

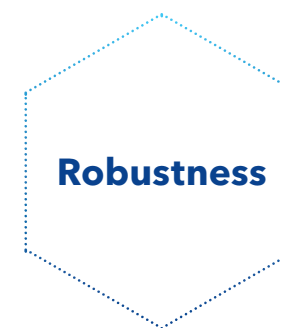
Range of applications	Separation and extraction ...
Pharmaceutical chemistry	Chiral compound (cis-trans phytol, steroids, peptides, antibiotics, etc.)
Food chemistry	Fatty acids, carbohydrate mixtures (sucrose/molasses or fructose/glucose, etc.)
Biochemistry	Phenylalanine, fermentation/cell culture products (citric acid, sugars, antibodies, enzymes, etc.)
Petrochemistry	C8-Hydrocarbon (xylene/toluene, etc.)

Why an 8 column setup is the best

Three reasons why more columns are better

The separation of biomolecules can also be done with 2, 3 or even 4 columns. But these systems are often very limited and cannot handle typical

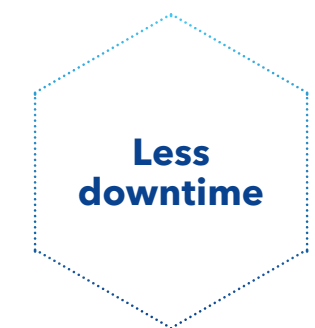
problems regarding process stability and flexibility as easy as the AZURA® SMB system can do. The 8 column setup has many advantages.



Other systems might be limited regarding their batch program adaption (wash+elution time \leq capture time), due to the flexible column distribution the length of every zone can be adjusted, even if there is a critical change in the feed concentration.



Sometimes additional purification steps are necessary to regenerate the column to keep their lifetime and potential at a maximum. Two and three columns systems often cannot handle this problem easily.



More columns mean longer system run time and less downtime. You will have up to four times less downtime due to column change. Use your working time most efficient.

Operation modes

Classical SMB chromatography

The standard configuration is designed to run classical SMB separations. This mode is perfect for the separation of binary mixtures, like sugars or e.g. pharmaceutical racemic mixture. The sep-

aration of a multi-component mixture into two different fractions is possible, too. This is typically used as one pre-purification step for very demanding separations.

Different zone configurations

In standard configuration every zone consists of two columns (2:2:2:2). To optimize the process, it might be useful to change this distribution. In case of a very effective regeneration of the solid and liquid phase in zone 1 and 4, the number of columns in these zones can be reduced. With a 1:3:3:1 configuration, a much higher productivity can be achieved. This system configuration can easily be adjusted via our software PurityChrom® MCC. The hardware does not have to be modified.

Open-/Closed-Loop

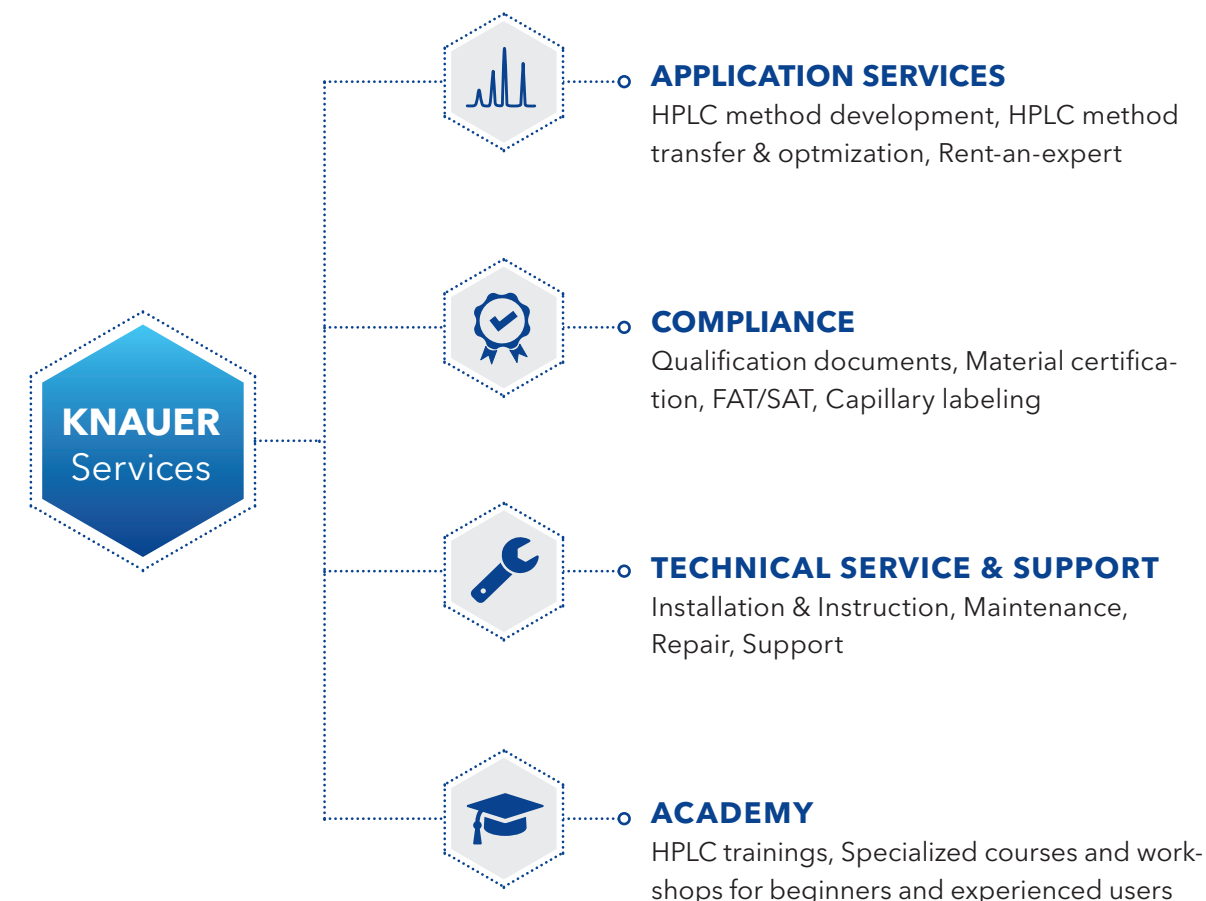
Every AZURA® SMB system can be switched between a Open- and a Closed-Loop mode. The Closed-Loop is the common SMB mode. In Open-Loop mode the SMB cycle is opened between the last column of zone 4 and the zone 1 pump. When to choose this mode?

- One impurity with very low retention time is in the sample (and eluent costs are low enough)
- To start/clean the system or change the eluent

Birch xylose converted to xylitol as sweetener



KNAUER Services



Contact us

All standard user instructions, helpful video tutorials, and a structured section of frequently asked questions is freely accessible on our web page www.knauer.net. If you need further support, our friendly Support team is happy to help you via e-mail, phone or Team Viewer. They will work with you personally until all issues are resolved.

Phone: +49 30 809727-111 (workdays 9-17h CET)
 Email: support@knauer.net

Application

Purification of xylitol by HPLC methods from fermented biomass

Process development

To optimize SMB parameters can be a very time consuming task, especially if a purity and a yield of more than 99% with a maximum productivity

Optimization of the analytical method

The analytical HPLC method has to be transferred to an isocratic method (Fig. 2). The stationary phase must be adapted to the later SMB process requirements too. In this case, the material was changed from 10 µm to 25-56 µm. Additionally the column dimensions were changed from 300 × 8 mm to 150 × 20 mm (Fig. 3)

is needed. In this application, xylitol was purified from fermentation mash of a fed-batch process.

Transfer into preparative scale

The next step is an overloading study based on the analytical method by using a column with the same dimensions of the prospective SMB process. Volume and mass overload are evaluated and adsorption isotherms are determined based on the retention times (Fig. 3). The received parameters are transferred into the appropriate SMB scale. Figure 4 shows where the chromatogram is split based on the calculated SMB parameter.

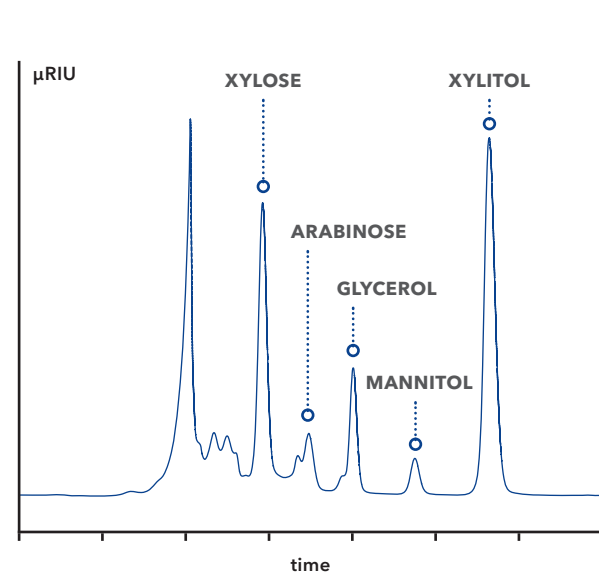


Fig.2: Analytical chromatogram of fermentation mash with identified sugar/sugar alcohols; 20 µL 1:2 dilution; Eurokat Ca 300 × 8 mm, 10 µm particle, 0.5 ml/min, 75°C, H₂O_{dd} isocratic

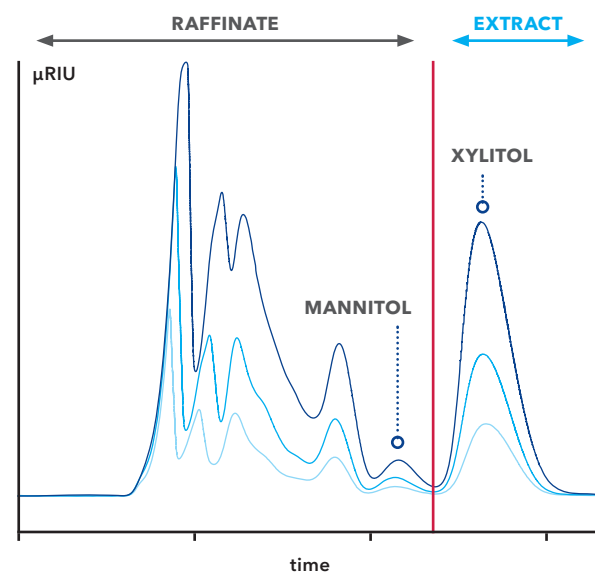


Fig.3: Semi-preparative chromatogram of fermentation mash; injection: light blue - 0.5 ml, blue - 1.0 ml, dark blue 2.0 ml; Eurokat Ca 150 × 20 mm, 25-56 µm particle, 4 ml/min 60°C, H₂O_{dd} isocratic

Evaluation of the SMB parameter

The adsorption isotherms (linear or Langmuir) can be easily inserted into the parameter wizard of our SMB software Purity-Chrom® MCC. The starting parameter will be calculated and transferred into the method automatically (Fig. 4).

Due to a contamination in the mixtures with nearly no interaction with the material, the SMB separation was done in an Open-Loop mode, so without solvent recycling.

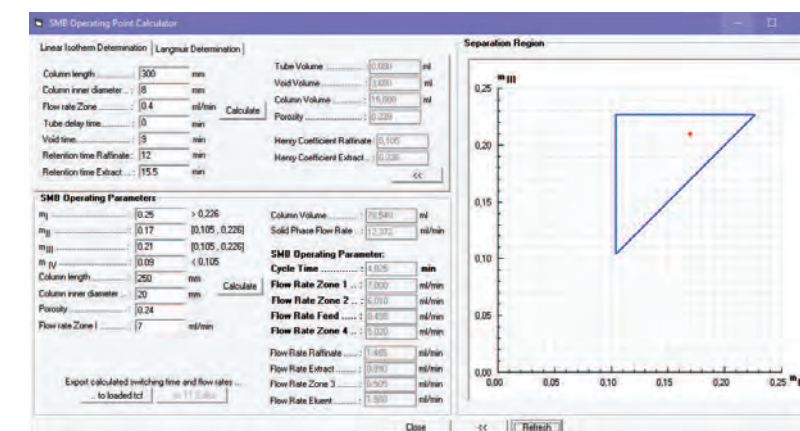
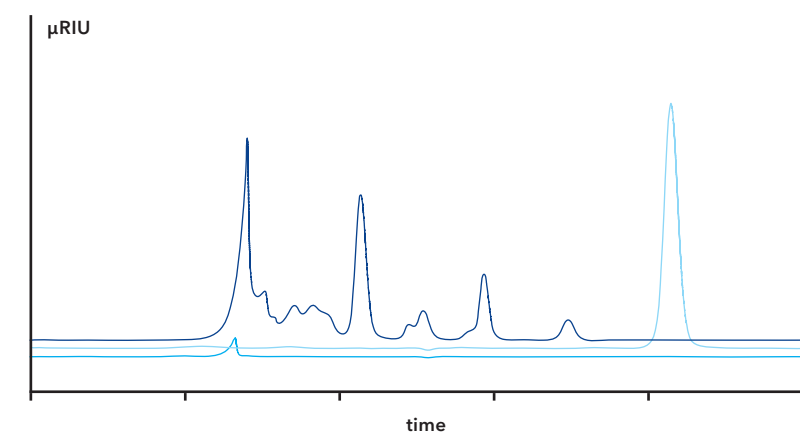


Fig.4: Parameter wizard in PurityChrom® MCC

Separation verification

Figure 5 shows an overlay of raffinate / extract and waste fraction of the 6th SMB cycle revealing a successful separation of the fractions, with 100% purity and recovery of xylitol.

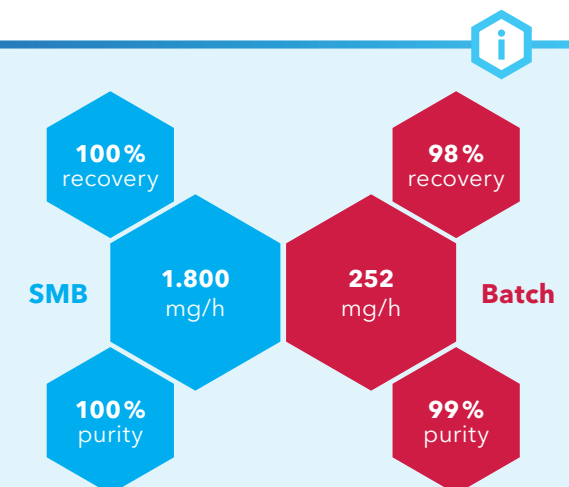


"Acknowledgement: This project has received funding from the European Union's Seventh Framework Program for research, technological development and demonstration under grant agreement no FP7-KB-BE-2013-7-613802."

Fig.5: Overlaid analytical chromatograms of raffinate (blue); extract / xylitol (light blue); waste (blue); 20 µL 1:2 dilution; Eurokat Ca 300 × 8 mm, 10 µm particle, 0.5 ml/min, 75°C, H₂O_{dd} isocratic

SMB versus Batch

The comparable batch separation (same conditions as seen in Fig. 3, dark blue chromatogram) offers nearly the same purity and recovery rate, but a significantly lower productivity of 252 mg/h. The throughput of the SMB process is with 1,8 g/h greater by the factor of seven than that of the batch process.



KNAUER customizable MCC systems

Special multi-column chromatography solutions

„KNAUER is the only company that could build a system according to our specifications“

Our focus is on the development of optimization and control strategies for multi-column processes. We needed a flexible system that is able to perform various multi-column processes on the highest level of technology. The hardware and the software must be flexible such that model-based optimization and control schemes can be tested on example processes. KNAUER offered the complete package for us: planning, designing, developing and manufacturing. The installation, instruction and support were very good. It was a pleasure working with KNAUER.



Prof. Dr. Ing. Sebastian Engell,
Head of Process Dynamics and
Operations
Technical University Dortmund

System components

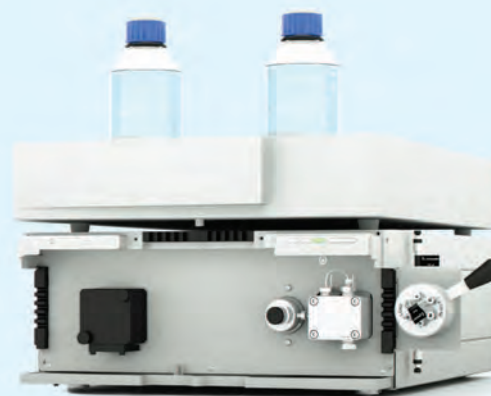
- 6 AZURA® Pumps P 6.1L HPG
- 2 AZURA® Pumps P 2.1S
- 2 AZURA® UV Detector UVD 2.1S
- 2 AZURA® Valve Drive V 2.1S
- 1 AZURA® CM 2.1S
- 24 AZURA® Valve Drive V 2.1S with multi-position valves
- Controlled via PCS by HiTec Zang

KNAUER builds up customized multi-column systems to your needs. We will support you by choosing the right devices, materials and control options.

Contact us:
sales@knauer.net



More KNAUER SMB solutions



Analytical system

Do you want to analyse your products directly? KNAUER offers very compact analytical systems. Take a look at our webpage.



Columns for purification

KNAUER offers a wide range of preparative LC columns. We have also a long time history in the production of columns especially for SMB chromatography. Our experts like to help you to make the correct choice from analytical up to preparative scale.



Application support

The development of a feasible SMB method is a very time consuming process. KNAUER can support you with the method development as well as SMB training.



AZURA® batch chromatography

Whenever separation tasks are changing frequently, a classical batch LC system can be the better choice. KNAUER AZURA® Prep and AZURA® Bio purification systems are as flexible and versatile as possible.

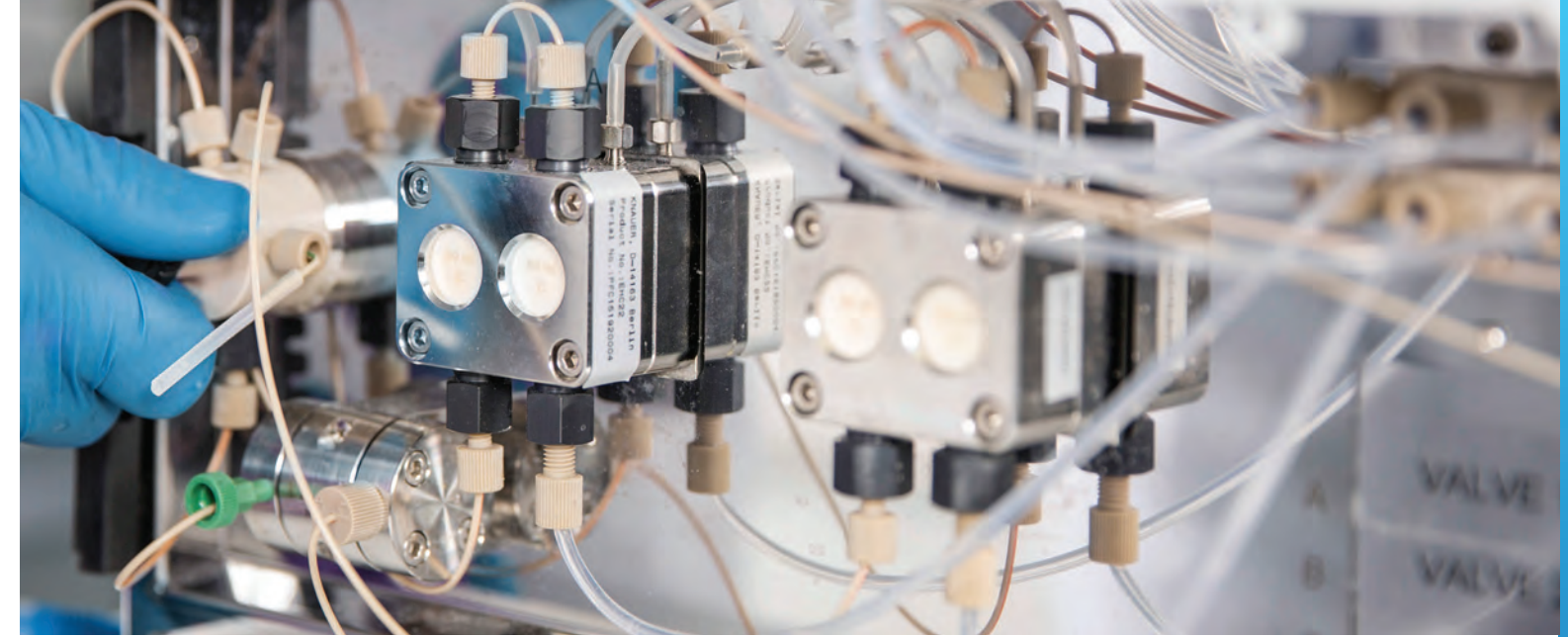
AZURA® Prep

AZURA® Prep was designed for flexibility and to comfortably handle large sample volumes. Easy to operate and maintain the AZURA® Prep systems are perfectly suited for the purification of

your products such as synthesis stages or active ingredients. The systems can be optimally adapted to the scale you need.



- Configure your system from injection to collection
- Choose between high or low pressure gradient systems from 50 to 1000 ml/min
- User-friendly and powerful software PurityChrom®

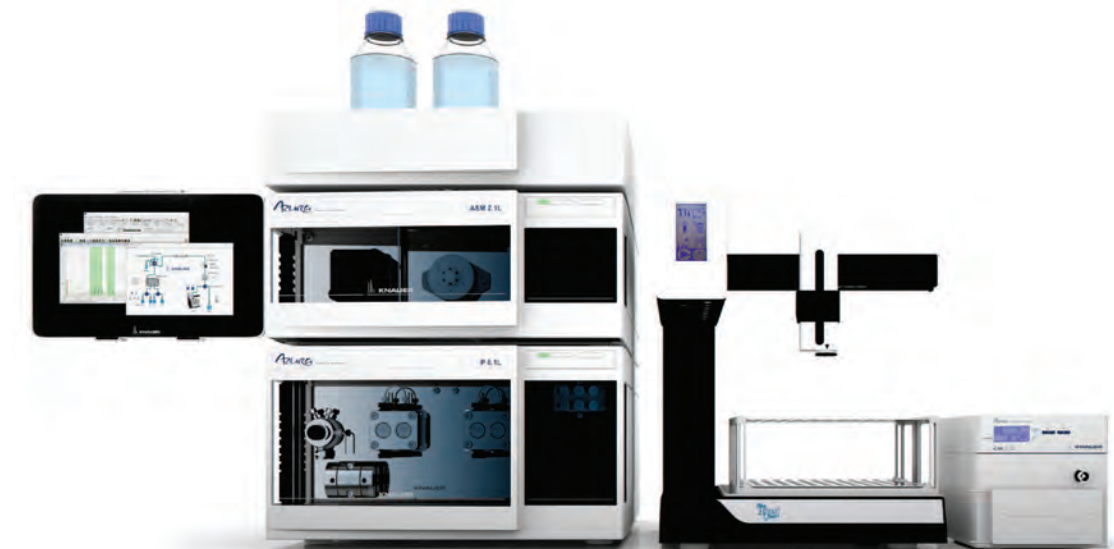


AZURA® Bio purification

Complete solution for FPLC on a minimum footprint: AZURA® Bio systems combine flexibility and reliability. The biocompatible AZURA® Bio is the perfect choice for your protein purification task.

Design your AZURA® Bio system to your needs. Multiple functionalities such as column switching, buffer and sample selection as well as fraction collection enable the user to automate their separation.

From simple to complex, from lab to pilot scale: Design your AZURA® FPLC system according to your purification task.



- Flexible and modular design
- Easy upscaling - up to 1000 ml/min
- Powerful FPLC software PurityChrom®
- Variety of great detectors to make your sample visible

Analytical
HPLC

Multi-Column
Chromatography,
SMB

Preparative
HPLC

FPLC

Osmometry

Dosing,
Metering,
Pumping

Detection

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